

University of Nottingham
UK | CHINA | MALAYSIA

PhD thesis

Atypical ovarian cycle length in dairy cows: Occurrence and effects on productivity and carbon emissions

Submitted to the University of Nottingham

By

Ibrahim Algherair

Supervisor(s): J. K. Margerison and P. C. Garnsworthy

Main abstract text length: 1,554 words

Total text length: 57,592 words

Keywords: Milk production, Fertility, Feed efficiency, Carbon equivalents

2022

Table of Contents

Page

Title: Atypical ovarian cycle length in dairy cows: Occurrence and effects on productivity and carbon emissions 1

I: Declaration 5

II: Acknowledgements 6

III: Acknowledgements for research funding and support 7

IV: Conflict of interest 7

V: Publications 7

VI: List of frequently used abbreviations 8

VII: List of Figures 10

VIII: List of Tables 12

IX: Abstract 15

Chapter 1: Literature review 19

1.0: Introduction 20

1.1: Importance of dairy cow fertility and key performance indicators 21

1.2: Individual milk yield of dairy cows and fertility 22

1.3: Dystocia, uterine disease, and fertility 25

1.4: Postpartum resumption of ovarian function and ovarian cyclicity 26

1.5: Monitoring ovarian cycles and fertility using milk progesterone concentrations 29

1.6: Monitoring oestrus activity 31

1.7: Conception and establishment, recognition, and maintenance of pregnancy 32

1.8: Body condition score at parturition and fertility 34

1.9: Postpartum change in body condition score and fertility 37

1.10: Postpartum and early lactation feed intake and fertility 39

1.11: Dietary and endogenous energy balance 41
1.12: Nutrient intake, feed efficiency and fertility 43
1.13: Conclusions 49
1.14: Aims and objectives of this thesis 50

Chapter 2.0: Experiment 1 51

The occurrence of atypical ovarian cycles and the effect on the calving interval, pregnancy, and replacement rates, and infertility-related carbon emissions from high-yielding dairy cows

2.1: Abstract 51
2.2: Introduction 52
2.3: Aim and objectives 54
2.4: Materials and methods 54
2.5: Measurements 56
2.6: Results 59
2.7: Discussion 60
2.8: Conclusions 62
2.9: Further work 62

Chapter 3.0: Experiment 2 63

Effect of atypical ovarian cyclicity on the fertility, milk yield, feed efficiency, and carbon dioxide equivalent of milk production from high-yielding dairy cows

3.1: Abstract 64
3.2: Introduction 64
3.4: Aim and objectives 65
3.5: Materials and methods 65
3.6: Measurements 68
3.7: Results 70
3.8: Discussion 75
3.9: Conclusions 79
3.10: Further work 79

Chapter 4.0: Experiment 3 81

Relationship between ovarian cycle length and fertility, milk yield, feed efficiency, calving interval, replacement rate, and potential carbon emissions from milk production

4.1: Abstract 82
4.2: Introduction 82
4.3: Aim and objectives 83
4.4: Materials and methods 84
4.5: Results 89
4.6: Discussion 94
4.7: Conclusions 97
4.8: Future work 97

Chapter 5.0: Experiment 4 99

Effect of atypical ovarian cyclicity on plasma metabolites, feed efficiency, replacement rates, and carbon equivalent of milk produced by high-yielding dairy cows

5.1: Abstract 100
5.2: Introduction 100
5.3: Aim and objectives 101
5.4: Materials and methods 101
5.5: Measurements 104
5.6: Results 106
5.7: Discussion 113
5.8: Conclusions 116
5.9: Further work 116

Chapter 6.0: General discussion 117
6.1: Carbon footprint of dairy production 118
6.2: Milk yield and assurance of atypical ovarian activity 118
6.3: Body condition score at parturition and atypical ovarian activity 119
6.4: Postpartum change in body condition score and atypical ovarian activity 120
6.5: Postpartum milk yield, feed intake and prolonged commencement of ovarian activity 121
6.6: Calving interval and replacement rate related carbon footprint 122
6.7: Outcomes compared with the objectives of the thesis 123
6.8: Study limitations and difficulties 124

References 125

I: Declaration

I declare that the thesis is the result of my own work which has been mainly undertaken during my period of registration for this degree at The University of Nottingham. I have complied with the word limit for my degree (as stated in the Quality Manual: https://www.nottingham.ac.uk/qualitymanual/research-degree-progs/pgr-prog-regs.aspx).

I confirm I have discussed any need for thesis embargo/restrictions with my supervisors.

Signature



Ibrahim Algherair

Date: 19/12/2022

Date: 21/05/23

II: Acknowledgements

I am grateful to Saudi Arabia Cultural Bureau (SACB) for the financial support for my PhD, without which this work could not have been accomplished. Also, I would give special thanks to my work at Saudi Arabia King Faisal University for giving me this opportunity to complete my PhD studies at one of the best universities in the UK.

I would like to express my deepest appreciation to the University of Nottingham for providing me with my supervisor, Dr Jean Margerison who made this work possible. Their guidance and advice carried me through all stages of writing my project. When I was under stress, they gave me the confidence and support to overcome these challenges. I have received generous support, constructive criticism, and invaluable guidance from them; help regarding statistical analyses and practical matters in animal sciences encouraged me to take the next step in becoming a scientist. I have benefited greatly from their knowledge, and I am extremely grateful that they took me on as a student and continued to have faith in me over the years. Moreover, I would give my deepest thanks to my second supervisor, Professor Phil Garnsworthy throughout my study, I received valuable advice and guidance, especially at the beginning of my PhD. I am also grateful for his support in participating in conferences and presenting papers. His encouraging words and thoughtful, detailed feedback have been very important to me. My previous supervisor George Mann, who I want to thank for your guidance when I was beginning my PhD and for your support in collecting data and doing lab work. It's a big, beautiful world out there, and adventure awaits! Wishing you all the very best in your retirement! I would like to thank all the farm staff, Jenifer Hankin and Neil Saunders for their technical support and giving me the opportunity to work as part of the team and helping me with my lab work, sample collection and explaining the aspects of lab work to ensure that I obtain the best quality outcomes. Many thanks to Wing Yee Kwong (Lydia) for teaching many elements of lab work and techniques and making me the best she possibly can. Also, I had the pleasure of collaborating with and offering mutual help to my teammates Lucy Ross, Chloe Geoghegan, and Kirsty Farnan.

On a personal note, I would especially like to thank my family. My wife, Alhanouf Alsharqi has been extremely supportive of me throughout this entire process and has made countless sacrifices to help me get to this point and constantly encouraged me throughout my research period. My children, Rakan and Raed have continually provided the requisite breaks from philosophy and the motivation to finish my degree with expediency. My mother, brothers, and sisters deserve special thanks for their continued support and encouragement. Without such a team behind me, I doubt that I would be in this place today. Their belief in me has kept my spirits and motivation high during this process. I wished my father was with me during these

moments and may God have mercy on his soul! Finally, I thank The University of Nottingham (UoN) from the bottom of my heart for giving me the opportunity to study in this welcoming place and for providing me an amazing study environment, and for the Nottingham community spirit and my friends who I would like to give them a special thank you for providing a pleasant and supportive atmosphere during my studies.

III: Acknowledgements for research funding and support

The provision of research animals and equipment are gratefully acknowledged from the University of Nottingham Centre of Dairy Science and Innovation (CDSI) and Centre for Innovation Excellence in Livestock (CIEL) for Experimental studies 1 to 4, and Harper Adams University (HAU) (Liam Sinclair) are acknowledged for the use of facilities and provision of data that was included alongside data from UoN in Experiment 3.

The funding of research support, provision of animals and consumables for: Experiment 1 are acknowledged from the UoN School of Biosciences (George Mann and Morag Hunter); Experiment 2 research support is acknowledged for commercial support from Phileo by Lesaffre (France) (Phil Garnsworthy and George Mann); Experiment 3 support is acknowledged for research from AHDB Dairy, from the AHDB Dairy Research Partnership on Animal Health Welfare and Nutrition (Kevin Sinclair, George Mann, Phil Garnsworthy, Liam Sinclair); Experiment 4 support is acknowledged for commercial research support from DSM (The Netherlands) (Jean Margerison) and research support from AHDB Dairy, from the AHDB Dairy Research Partnership on Animal Health Welfare and Nutrition (Kevin Sinclair, George Mann, Phil Garnsworthy).

IV: Conflict of interests

There are no conflicts of interest involved in the completion of the research, data analysis and interpretation of the results presented in this thesis.

V: Publications

Algherair, I. Saunders, N., Goodman, J., Garnsworthy, P. C., Margerison, J. K and Mann, G et al. (2022). 42: Effect of live yeast (Saccharomyces cerevisiae) on milk yield, feed efficiency and reproduction of high-yielding dairy cows. Animal –Science Proceedings, 13, 23. The Role of Animals in Human and Planetary Health, British Society of Animal Science. Nottingham, UK, 11 – 14 April 2022.

Algherair, I., Garnsworthy, P. C., Margerison, J. K and Mann, G (2021). 164: Effect of atypical reproductive cycles on key performance indicators of reproductive efficiency in Holstein Friesian dairy cattle, Animal - Science Proceedings, 12, 134.

VI: List of frequently used abbreviations

AA: Amino acid
ACL: Atypical ovarian cycle length
AFC: Age at first calving
AYR: All year round
AI: Artificial insemination
BCS: Body condition scoring
BCSP: Body condition score at parturition
BHBA: Beta hydroxyl butyric acid
CI: Calving interval
CL: Corpus luteum
CNS: Central nervous system
COD: Cystic ovarian disease
CO_2-eq.: Carbon dioxide equivalent
CR: Conception rate
DMI: Dry matter intake
EB: Energy balance
ECM: Energy corrected milk
ELISA Enzyme linked immunosorbent assay
FA: Fatty acid
FCM: Fat corrected milk
FPCM: Fat and protein corrected milk
FE: Feed efficiency
FME: Fermentable metabolizable energy
FSH: Follicle-stimulating hormone
GB: Glucose balance
GH: Growth hormone
GHBP: Growth hormone binding protein
GHG: Greenhouse gas
GHR: Hepatic growth hormone receptor
GLUTs: Glucose transporters
GnRH: Gonadotropin-releasing hormone
HPG: Hypothalamus pituitary gonadotropin
IGFBPs: IGF-binding proteins
IGF-I: Insulin-like growth factor-I
IGF-II: Insulin-like growth factor-II
KPIs: Key performance indicators

LH: Luteinising hormone

MEC: Mammary myoepithelial cell

M/D: Energy density (ME/DMI) of the diet

mRNA: Messenger RNA

MS: Milk solids (milk fat + milk protein kg/d)

MY: Milk yield

NEB: Negative energy balance

NEFA: Non-esterified free fatty acids

NFC: Non-fibre carbohydrates (starch, sugar, and pectin) are calculated by subtracting the crude protein, neutral detergent fiber, fat, and ash from 100%.

P_4: Progesterone

PGE_2: Prostaglandin E_2

$PGF_{2\alpha}$: Prostaglandin F_2alph

£PLI: Production lifetime performance

pp: Postpartum

PPI: Postpartum interval

PR: Pregnancy rate

RR: Replacement rate

TAG: Tri acyl glyceride

TCL: Typical ovarian cycle length

VII: List of Figures Page

Fig 1.1: Changes in milk production (lbs) and pregnancy rate (%) of US Holstein cattle over six decades (Brito et al. 2021) data source: Council on Dairy Cattle Breeding website (July 2020; www.uscdcb.com) 18

Fig. 1.2: Schematic presentation of the reasons for the major problems contributing to low fertility in dairy cows in the postpartum period (Walsh et al. 2011) 21

Fig 1.3: Resumption of dominant follicles and ovarian cycles during the postpartum period in dairy and beef suckler cows not nutritionally stressed (Crowe and Mullen, 2013, from Crowe, 2008) 23

Fig. 1.4: Schematic depiction of the pattern of secretion of progesterone, FSH, LH, $PGF_{2\alpha}$, and oestradiol (estradiol) during the oestrous cycle in cattle (Toledo-Alvarado et al. 2018) (Dioestrus (Diestrus): High-progesterone (diestrus-HP) ranging from −10 to −4 d; Proestrus: ranging from −3 to −1 d; Oestrus at d 0; Metoestrus from 1 to 2 d; Dioestrus: increasing-progesterone (dioestrus-IP) 3 to 10) 25

Fig. 1.5: Schematic presentation of the most important factors affecting the establishment of pregnancy in dairy cows (Walsh et al. 2011) 29

Fig. 1.6: Schematic representation of mechanisms through which nutrient intake and adipose tissue affect reproductive function (Garcia-Garcia, 2012) 34

Fig. 1.7: Feedback-regulation systems that control the reproductive axis (shaded is A) and the somatotropic axis (shaded is B) interact at several levels and thus link nutritional and metabolic inputs into the reproductive process (Chegas et al. 2007) 35

Fig. 1.8: Schematic summary of the effect of energy shortage / negative energy balance (NEB) on fertility through the effect on the liver, ovaries, and uterus (Nigussie, 2018) 38

Fig. 1.9: Weekly mean supply (■) and demand (■) for glucogenic carbon and glucose balance (□) of high milk-yielding dairy cows between parturition and 105 d pp (Habel et al. 2022) 41

Fig 3.1: Schematic of the proportion of cows that had atypical (ACL) and typical (TCL) cycle lengths from cows that were offered no yeast (NY) and yeast (+Y) in the partial mixed ration

Fig 4.1: Schematic of the proportion of cows that had atypical (ACL) and typical (TCL) cycle lengths from the University of Nottingham (UoN) and Harper Adams University (HAU) experimental sites

Fig 5.1: Schematic of the proportion of cows that had atypical (ACL) and typical (TCL) cycle lengths from differing experiments completed in 2016/17 and 2018/19 at the University of Nottingham (UoN)

VIII: List of Tables Page

Table 1.1: Key performance indicators for all year-round (AYR) calving dairy herds 19

Table 1.2: Recommended body condition score and change in body condition score for dairy cows in Great Britain 35

Table 2.1: Application of milk progesterone (P4) concentrations used to categorise atypical ovarian activity of lactating dairy cows 54

Table 2.2: Partial mixed ration ingredients and composition of mixed ration and pelleted concentrate offered at milking 57

Table 2.3: Mean (±SE) parity, body condition (Range) and postpartum interval to conception, conception and pregnancy rate and calving interval of high yielding dairy cows that had atypical and typical luteal cycles 59

Table 2.4: Carbon equivalents related to herd replacements, calving interval (±SE) and infertility of high yielding dairy cows that had atypical and typical luteal cycles 60

Table 3.1: Partial mixed ration ingredients and composition of mixed ration and pelleted concentrate offered at milking 67

Table 3.2: Mean reproduction performance (± se) of dairy cows that had atypical and typical postpartum oestrus cycle length 71

Table 3.3: Mean yield and composition (± se) of milk of dairy cows that had atypical and typical postpartum oestrus cycle length 72

Table 3.4: Mean dry matter intake (DMI) (± se), rumination and feed efficiency of dairy cows that had atypical and typical postpartum oestrus cycle length 73

Table 3.5: Range and mean body condition score BCS (± se) of dairy cows atypical and typical postpartum oestrus cycle length 74

Table 3.6: Mean of live weight (± se) of dairy cows atypical and typical postpartum oestrus cycle length 74

Table 3.7: Carbon dioxide equivalent (± se) of dairy cows that had atypical and typical postpartum oestrus cycle length 75

Table 4.1a: Composition of mixed ration and concentrate feeds offered to dairy cows that had atypical and typical oestrus cycles 86

Table 4.1b: Nutrient concentrations of mixed ration and concentrate feeds offered to dairy cows that had atypical and typical oestrus cycles 87

Table 4.2: Mean reproductive performance (± se) of dairy cows that had atypical and typical oestrus cycle length 89

Table 4.3: Pearson correlation R values[1] between ovarian cycle length and key performance indicators of fertility 89

Table 4.4: Range and mean of body condition score BCS (± se) of dairy cows that had atypical and typical oestrus cycle length 90

Table 4.5: Pearson correlation R values[1] between ovarian cycle length and body condition score and postpartum change in body condition score 91

Table 4.6: Pearson correlation R values[1] between ovarian cycle length and live weight and postpartum liveweight change 91

Table 4.7: Mean yield and composition (± se) of milk from dairy cows that had atypical and typical oestrus cycle length 92

Table 4.8: Mean feed intake (DMI) (± se) and feed efficiency of dairy cows that had atypical and typical oestrus cycle length 93

Table 4.9: Person correlation R values[1] between ovarian cycle length and milk yield, dry matter intake (DMI) feed efficiency 93

Table 4.8: Mean replacement rate, additional days of calving interval and related carbon equivalent (± se) of dairy cows that had atypical and typical oestrus cycle length 94

Table 4.9: Person correlation R values[1] between ovarian cycle length and replacement rate (RR), days over a 365-d calving interval (CI) and CO2-eq. related to CI, RR and infertility 94

Table 5.1: Partial mixed ration ingredients and composition of mixed ration and pelleted concentrate offered at milking 103

Table 5.2: Mean reproduction performance (± se) of dairy cows that had atypical and typical postpartum ovarian cycle length 106

Table 5.3: Person's correlation R values[1] between postpartum ovarian cycle length and mean reproduction performance 106

Table 5.4: Mean yield and composition (± se) of milk of dairy cows that had atypical and typical postpartum ovarian cycle length 107

Table 5.5: Person's correlation R values[1] between postpartum ovarian cycle length and mean milk yield and composition 108

Table 5.6: Mean dry matter intake (DMI) (± se), rumination and feed efficiency of dairy cows that had atypical and typical postpartum ovarian cycle length 108

Table 5.7: Range and mean of body condition score BCS (± se) of dairy cows atypical and typical postpartum ovarian cycle length 109

Table 5.8: Person's correlation R values[1] between postpartum ovarian cycle length and mean milk yield and composition 110

Table 5.9: Mean beta-hydroxy butyrate (BHB) (± se) concentrations of dairy cows that had atypical and typical postpartum ovarian cycle length 110

Table 5.10: Mean non-esterified free fatty (NEFA) acid, plasma glucose and insulin (± se) concentrations of dairy cows that had atypical and typical postpartum ovarian cycle length 111

Table 5.11: Mean plasma and urea and haptoglobin concentrations (± se) of dairy cows that had atypical and typical postpartum ovarian cycle length 112

Table 5.12: Carbon dioxide equivalent (± se) of dairy cows that had atypical and typical postpartum ovarian cycle length 113

Table 5.13: Person's correlation R values[1] between postpartum ovarian cycle length and mean milk yield and composition 113

Atypical ovarian cycle length in dairy cows: Occurrence and effects on productivity and carbon emissions

IX: Abstract

Enteric fermentation by ruminants contributes to 30% of global livestock greenhouse gas (**GHG**) emissions (FAO, 2018), making ruminants one of the largest contributors per unit of feed intake. Dairy production has been cited as a major GHG emitter contributing to climate change, however increasing the milk yield (**MY**) per cow has been successful in increasing feed efficiency (**FE**) and reducing enteric GHG emissions per litre of milk production. This is essential in maintaining global warming < 1.5°C up to 2030 and net zero by 2050, however the potential associated decline in pregnancy rate (**PR**) and infertility is a main factor that may contribute to GHG emissions, mainly due to the decline in the length of productive life of dairy cows. Survey data and modelling has been used to evaluate carbon emissions (**CO_2-eq.**) per day of calving interval (**CI**) greater than 365 d and per percentage increase in dairy herd replacement rate (**RR**). This thesis aims to to evaluate the postpartum ovarian activity to classify dairy cows that had typical and atypical ovarian cycle length and evaluate the fertility and production data and calculate the impact of the length of the CI and RR on CO_2-eq. of dairy production from Holstein Friesian (**HF**) dairy cows. The thesis presents four experiments (Expt. 1 to 4) that evaluate the occurrence of atypical postpartum ovarian cycle length (**ACL**) and compare the effect of ACL with typical cycle length (**TCL**) on fertility using milk progesterone (P4) concentrations to assess postpartum (**pp**) ovarian cycles. This thesis compares the effect of ACL on the pp interval (**PPI**) to conception, CI and PR and RR, and apply previous UK estimates of CO_2-eq. for each addition day over a CI of 365 d and additional percent of RR to compare the effect of ACL and TCL on CO_2-eq. in relation to CI, RR, infertility and FE.

Expt. 1 set out to collect a large data set from 230 HF dairy cows to estimate the occurrence rate of ACL, assess related factors and calculate the effect of ACL on CO_2-eq. in relation to CI and RR. Cows with ACL (31%) were of a greater parity (P=0.032) and had a greater PPI to first progesterone (**P4**) rise (P<0.001), lower CR to the first insemination (P<0.034), longer PPI to conception (P<0.001) and CI (P<0.001), while PR and BCS at parturition did not differ between cows that had ACL and TCL. Occurrence of ACLs increased the carbon footprint (CO_2-eq) produced by additional RR (P<0.001) and a CI above 365 d and 375 d increased the CO_2-eq. related to CI (P<0.001), and infertility (P<0.001), which was a combined effect on CO_2-eq. related to additional herd replacements and CI. It was concluded that 31% of high-yielding dairy cows exhibited ACLs, which increased the PPI to the first insemination, lowered the conception to the first insemination, and increased the PPI to conception and CI of dairy

cows. Infertility substantially increased the CO_2-eq of dairy cows due to cows that had ACLs having a longer PPI to first P4 rise, first insemination and conception, which increased the CI. However, this need to be considered in relation to other factors such as pp change in BCS, MY and FE.

Expt. 2 aimed to assess 49 high MY HF dairy cows to evaluate the occurrence and effect of ACL and TCL on MY and FE and found that ACL occurred in 31% of cows and cows that had ACL had greater MY and FE than cows with TCL. Cows that had ACL had a greater PPI to first progesterone rise (P=0.003) and to conception and CI (P=0.005) at 160 d pp, but not 130 d pp which showed poor fertility in cows with ACL and TCL at 130 d pp. The CR and PR did not differ at 130 d and 160 d pp between cows with ACL and TCL. Dairy cows that exhibited ACLs had greater fat and protein corrected milk (**FPCM**) (P<0.001) and milk protein (**MP**) yield (P<0.001) and were more FE (FPCM P=0.015; MP P=0.002) but had greater CO_2 eq. emissions mainly due to a greater CI (P= 0.010). Body condition score (BCS) at parturition and pp change in BCS did not differ between cows that had ACLs and TCL and were well within industry recommendations. It was concluded that the high MY dairy cows in this study that exhibit ACL had longer PPI to conception and CI at 160 d pp, which continued not to differ and was not related to BCSP and pp change in BCS. Cows with ACL had greater FPCM, MP yields, and FE (FPCM and MP yield), while the extended CI increased CO_2 eq. of milk production, which disagreed with the results found in Expt. 1. However, this study had assessed a relatively small data set, especially for evaluating fertility and relationships between ACL and other factors such as MY and FE using correlation. The aim going forwards was to seek out larger data sets that had assessed milk P4 concentrations to monitor pp ovarian activity and use these to assess the effect of ACL and the relationship between ACL and other factors that lower CO_2 eq. of milk production, such as MY and FE.

Expt. 3 used a data set that had been collected from 88 HF dairy cows based on two differing sites that had had pp milk P4 concentrations measured. This data set was used to assess the effect of site and previous diet treatment on the occurrence of ACL, neither of which had an effect on ACL. The data set was then used to assess the occurrence and effect of ACL on fertility and the MY and FE of cows with ACL and TCL. ACL continued to be relatively common occurring in 30% of cows and increased ovarian cycle length (P=0.047), postpartum interval (**PPI**) to first milk P4 rise (P<0.001), PPI to the first insemination (P=0.006), and the number of inseminations per conception (P=0.002) but had no effect on the CR to first service, PPI to conception, PR and CI. Moderate correlations existed between ovarian cycle length and the PPI to the first AI, the no. of inseminations/conception, and conception rate. There was no difference in MY, and FPCM yields, BCS or LW, and pp change in BCS and LW, DMI, FE,

RR, and CO2-eq. related to RR, CI and infertility between cows that had ACL and TCL. Correlation analysis showed low strength in the relationship between ovarian cycle length and MY, FE and CI or RR. However, cows produced substantial amounts of CO_2-eq. due to an extended CI and RR, which was highly correlated with RR. It was concluded that the occurrence of ACL was relatively common, delaying the PPI first P4 rise and insemination and increasing the number of inseminations per conception, which was not related to greater MY, pp BCS loss and FE. However dairy cows in this study had substantial amounts of CO_2 eq. attributed to a greater RR and the provision of herd replacements. The effect of ACL and its relationship with MY differed between Expt. 2 and this experiment, with MY and FE being greater in cows that had ACL in Expt. 2, but not in this study. A further large-scale study was required to assess the effect of ACL on MY and FE, that allowed the relationship between factors and assess plasma metabolites that may be related to ACL.

Expt. 4 aimed to use 97 cows from a single site to assess the relationship between ACL and MY, FE and CO_2-eq. of milk production and elucidate any relationships between ACL and plasma metabolites. Cows that had ACL (28.3%) had a longer PPI to first progesterone ($P<0.001$), cycle length ($P<0.001$) and tended to have a longer PPI to conception and CI ($P=0.053$), while PR and RR did not differ between ACL and TCL at 130 d pp. Cows that exhibited ACLs tended ($P=0.090$) to have greater MY and milk fat concentrations, but FCM, FPCM and milk protein yields and FE did not differ from cows that had TCL. Plasma NEFA, BHB, insulin, glucose and haptoglobin, body condition score at parturition and pp change in BCS did not differ between cows that had ACLs and TCLs and were within industry recommendations. Cows with ACL had more addition days over a CI of 365 d ($P=0.046$), related RR ($P=0.046$) and CO2 eq. related to an extended CI compared with TCL, while RR contributed substantially to the CO2 eq. It was concluded that a substantial proportion of cows exhibited ACL, which cows tended to have greater MY and was not affected by BCS and plasma metabolites and had no effect on ECM, FPCM, milk protein yields and FE. The CO2 eq. of milk production was increased by extended CI and was associated with higher herd RR.

Overall, it was concluded that ACL occurred in between 28 and 31 % of HF dairy cows and ACL increased the PPI to first P4 rise and extended the CI. ACL was not related to body condition score (**BCS**) at parturition and loss of BCS following parturition that were well within industry standards of 2.5 to 3.0 and < 0.5 up to 60 d pp (0 to 5-point scale, with 0.25 increments) respectively. ACL was not related to plasma glucose, insulin, NEFFA and BHB concentrations. Cows that had ACL had (Expt. 2) ($P<0.001$) and tended ($P=0.090$) (Expt. 3) to have higher MY and FE and improvement of DMI during early lactation (first 14 to 24 d of lactation) may lower the occurrence of ACL and reduce the PPI to ovarian activity and CI of

dairy cows. Increases in the CO_2-eq. of milk production were related to ACL and an extended CI and greater herd RR. Lowering dairy herd RR shows the greatest potential to reduce CO_2-eq. from milk production.

Keywords: Fertility, Replacement rates, Milk yield, Feed efficiency, Carbon equivalents

Chapter 1

Literature review

1.0: Introduction

Enteric fermentation by ruminants contributes to 30% of global livestock emissions (FAO, 2018). Ruminants have been one of the largest contributors per unit of feed intake (DEFRA, 2021). The current world population has been forecast to reach 9.8 billion by 2050 (FAO, 2020) and the dairy industry needs to continue to provide millions of them with livelihoods (OECD-FAO, 2022) and healthy nutrition (Davies et al. 2018; Mottet et al. 2018; ADA, 2019; Poppit, 2020), especially for the development and maintenance of strong bones and teeth (NHS, 2022). In the next decade, world milk production has been projected to grow faster than most other agricultural commodities, at 1.8% p.a., to 1 060 Mt by 2031, through greater individual cow milk yield (MY) and the number of dairy cows (1.1% p.a.) in regions with low MYs, such as Sub-Saharan Africa and countries such as India and Pakistan (OECD-FAO, 2022), while the USA, Europe, and UK are forecast to continue to consolidate the number of dairy cows and herds while increasing individual MY (Margerison, 2022). Dairy production has been cited as a major greenhouse gas (GHG) emitter that contributes to climate change (Caro et al. 2014) and life cycle analysis (LCA) has shown that the main determinants of GHG emissions in industrialized countries, not considering carbon emissions from land use/land use change, is methane (CH_4) produced by enteric fermentation in the rumen, along with GHG emissions arising from the production, processing, transport, and storage of feed and the rearing of replacement animals (Flysjö et al. 2011; Wolf et al. 2017).

Increasing MY per cow has been successful in achieving greater feed efficiency (FE) and the dilution by spreading enteric GHG emissions related to maintenance over more litres of MY (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014), which is essential in maintaining global warming < 1.5°C up to 2030 and achieving net zero emissions by 2050 (UN, 2022). However, increased MY per cow has however been associated with a decline in fertility (FAO, 2005; Butler, 2003; Royal et al. 2000; Moore and Thatcher, 2006; Bello et al. 2012), reductions in the length of productive life (LPL) (Knaus, 2009) and greater GHG emissions related to less frequent calving (Bell and Wilson, 2018) and rearing of replacement animals (Zehetmeier et al. 2014; Bell et al. 2015) and may even improve the profitability of milk production (Gröhn and Rajala-Schultz, 2000; Horn et al. 2012; De Vries, 2017). The effectiveness of this approach is declining (VandeHaar, 1998; Reynolds et al. 2011; Ripple et al. 2014; Pickering et al. 2015; VandeHaar et al. 2016). While the genetic selection of low CH_4 emitting cows can be an effective and sustainable approach to reduce GHG emission from dairy cattle (Garnsworthy et al. 2012; Lassen and Difford, 2020) there is a lack of consensus regarding the optimal CH_4 traits (de Haas et al. 2017; Løvendahl et al. 2018) and further reductions in GHG emissions can come from improvements in animal health and fertility (Bell and Wilson, 2018) and the application of livestock production systems

that lower GHGs from livestock production systems (OECD-FAO, 2022). This requires a greater understanding of the factors that enhance livestock, feed, and land use and lower the production of GHGs and environmental impact of livestock production.

1.1: Importance of dairy cow fertility and key performance indicators

Dairy cow selection strategies have achieved greater FE by focusing largely on the Holstein Friesian, which had a greater emphasis placed on MY at the expense of other traits such as fertility, health, and longevity, raising concerns regarding the resilience and sustainability of the industry (Bell and Wilson, 2018; Clay et al. 2020). In terms of poor cow longevity (Bell et al. 2010) infertility continues to be the main challenge (Kossaibati and Esslemont, 1997).

Table 1.1: Key performance indicators for all year-round (AYR) calving dairy herds

Key performance indicators	Performing well (Top 5%)	Room to improve (Top 25%)	Performance reviews
Pregnancy rate (%)	>25	25 to 14	5 to 14
Age at first calving (mo.)	23 to 24	24 to 27.5	27.5 to 30
Lifetime milk yield, L/d	>19	19 to 12.5	8 to 12.5
Genetic merit [1], percentile	< 5 th	5 th to 50 th	> 50 th
Overheads [2], ppl	7 to 9	9 to 12.5	12.5 to 20
Milk yield from forage, L	3,000 to 2,000	2,000 to 1,500	1,500 to 1,000
Milk solids from forage, kg	230 to 150	150 to 115	115 to 70

Amended from AHDB, 2022: https://ahdb.org.uk/all-year-round-calving-key-performance-indicators

[1] - Production lifetime performance (£PLI) [2] - Excl. rent and finance

Improvements in dairy herd fertility has the potential to lower methane (10 to 11%) and ammonia (9%) emissions. Fertility is of great financial and environmental importance to the sustainability of the dairy industry because an extended calving interval (**CI**) has been shown to increase GHG emissions by 15 kg of carbon dioxide equivalent (**CO2-eq.**) / cow and 23 kg CO2-eq. / kg milk solids (**MS**) and cost approx. £2.80 / d for each day over the traditional annual 365 d CI, while the culling/replacement rate costs approx. £13.50 / % and increases CO2-eq. emissions by 50 kg/cow and 91 kg/kg MS (Bell and Wilson, 2018). Infertility has a major effect on the number of heifer replacements required (Garnsworthy, 2004), and the levy-

funded body in the UK that services the agricultural industry of Great Britain has key performance indicators (**KPIs**) for dairy herds that directly relate to seasonal and all year round (**AYR**) calving (AHDB, 2022) (Table 1.1). In AYR calving herds, the pregnancy rate (**PR**), age at first calving (**AFC**), and lifetime MY have been important KPIs, which have been directly affected by infertility. In seasonal calving systems, milk yields per cow have been lower (Margerison, 2022), and considerable focus on ensuring cows calve annually in line with spring grass production (Butler and Smith, 1989; Margerison, 2011, 2022) and/or autumn housing. This has been achieved by the application of slightly differing KPIs such as PR / 'in calf' rate, but at 3 and 6 weeks following the planned start of mating to ensure cows calve over a short time period ('tight block'), while the overall PR is more commonly expressed as an empty (**MT**) rate, describing the potential loss of non-pregnant cows that have been most likely culled, but some may be sold from spring into autumn calving herds and very few may be maintained as 'carry-overs' from one year to another (Margerison, 2022).

Milk yield and AFC have been the basis of production lifetime performance (**£PLI**) (Table 1.1) and in the UK AFC has been well above the 22 to 24 mo. recommended (Heinrichs, 1993; Hoffman, 1997; Gabler et al. 2000; Margerison and Downey, 2005; Cooke et al. 2013; Eastham et al. 2018) at 26.9 (Sherwin et al. 2016) to 29.1 mo. (Eastham et al. 2018) and ranges between 24.5 and 31 (Brickell et al. 2009; Ojango and Pollott, 2001; Froidmont et al. 2013) and 35.9 (Sherwin et al. 2016) to 40.9% of heifers calve for the first time >30 mo. of age (Eastham et al. 2018). A greater AFC has been associated with animals laying down body condition and having a greater BCS at parturition (**BCSP**) and calf birth weight increasing dystocia, which was followed by more uterine disease and longer pp CI, greater initial, peak, and whole lactation MY (Atashi et al. 2021). Lower PR, longevity and lifetime productivity (Nilforooshan and Edriss, 2004; Wathes et al. 2014, Zavadilová and Štípková, 2013; Adamczyk et al. 2017; Eastham et al. 2018; Zavadilová L and Štípková et al. 2020) has been related to a greater AFC. While poor heifer growth rates, due to ill health and inadequate nutrition, delay puberty and negatively affect fertility and subsequently increase the AFC (Wathes et al. 2018). In dairy production heifer rearing costs represent the second largest expense, following feed costs, accounting for approx. 15 to 20% of the total dairy farm costs (Boulton et al. 2015), which has been increased by dairy cow culling rates and CI. Lowering the AFC has been shown to increase the margin between income from MY and rearing costs per heifer by $41.5 (£34.41) for an AFC of 24 mo. and $24 (£19.90) for an AFC of 22 mo. (Pirlo et al. 2000) compared with an AFC of 26 mo.of age. Infertility in dairy cows has a significant financial impact in all dairy systems affecting MY and income from milk sales (Royal et al. 2000; Plaizier, Lissemore, Kelton and King, 2010; Kasimanickam et al. 2014) because less regular CI lifetime MY (Royal et al. 2008) generated from a greater number of lactations

and proportion of time spent in peak / early lactation (Margerison, 2022). This process begins with the involution of the uterus and postpartum interval (**PPI**) to the resumption ovarian cyclicity (Lovendahl and Chagunda, 2010; Walsh et al. 2011), which require follicular growth and the development of viable oocytes prior to oestrus and ovulation, followed by fertilisation, conception and the implantation, growth, development, and maintenance of a viable foetus (Walsh et al. 2011) and disturbances in these can result in poor fertility (Leroy et al. 2008).

1.2: Individual milk yield of dairy cows and fertility

There have been currently more than 270 million dairy and dual-purpose cows producing milk globally, however more than 40% of the total world milk has been produced by high-yielding dairy cows in 33 countries (Margerison et al. 2022) focused on increasing the individual MY of cows, which has been successful in increasing the economic, feed and environmental efficiency of milk production (Knapp et al. 2014) and increased MY per se reduces methane emission per unit milk yield (Reynolds et al. 2011), while ensuring dairy food security. Equally, the dairy industry in developed countries continues to increase the individual milk yield of dairy cows, largely due to the adoption of pure and cross-bred Holstein Friesian genetics and concurrent improvements in nutrition (OECD-FAO, 2022). In the UK the number of dairy cows and herds continues to decrease, while herd size and individual MY continues to increase (Margerison, 2022) and such structural changes have been associated with a decline in fertility (FAO, 2005; Butler, 2003; Royal et al. 2000; Moore and Thatcher, 2006; Bello et al. 2012). Fertility has been especially challenging for dairy cows because they have been one of the few mammals that lactate while becoming pregnant, which creates a direct competitive relationship between the priority for nutrients for milk synthesis (Lucy, 2003; Leroy et al. 2010a) and investment in the reproduction of the next generation (Silvia, 2003) and this increases the risk of ovarian dysfunction (Wiltbank et al. 2002; Lopez-Gatius, 2003; Leroy et al. 2008). Greater MY has been successful in diluting the nutrient requirements for maintenance (Capper and Bauman, 2013) and increasing FE (VandeHaar and St-Pierre, 2006), which increases the amount of nutrients partitioned towards MY and extends this priority beyond early lactation (Leroy et al. 2010a), defined in fertility terms as < 70 d pp (Chiba, 2009), which coincides with first insemination, conception rate (**CR**) and PR required to achieve regular calving CI associated with greater MY (Royal et al. 2008).

In the USA and UK genetic selection indices for Holstein-Friesian cows historically emphasised MY and placed little emphasis on characteristics connected with fitness, such as health, fertility and conformation traits that have been related to longevity (Pyman, 2006). An extensive amount of literature has shown that cows that have greater MY generally have poorer fertility (Spalding et al. 1975; Berget al. 1981; Lean et al. 1989; Butler, 2000; Westwood

et al. 2002; Pryce et al. 2004), and that genetic selection for increased MY can reduce fertility (Hageman et al. 1991; Buckley et al. 2000; Horan et al. 2005; Pollott and Coffey, 2008; Eggar-Danner et al. 2015). Greater MY has specifically been found to delay the pp commencement of luteal activity (Butler, 2000; Reist et al. 2000; Martin et al. 2015) and PPI to conception, lower conception to first service and overall CR and PR, while increasing the services required per conception (Castillo-Juhave beenz et al. 2000; Pryce et al. 2004; Inchaisri et al. 2010; Andersen et al. 2011). Increases the GHG have been associated with a greater CI (Bell and Wilson, 2018) and number of herd replacements required (Zehetmeier et al. 2014; Bell et al. 2015).

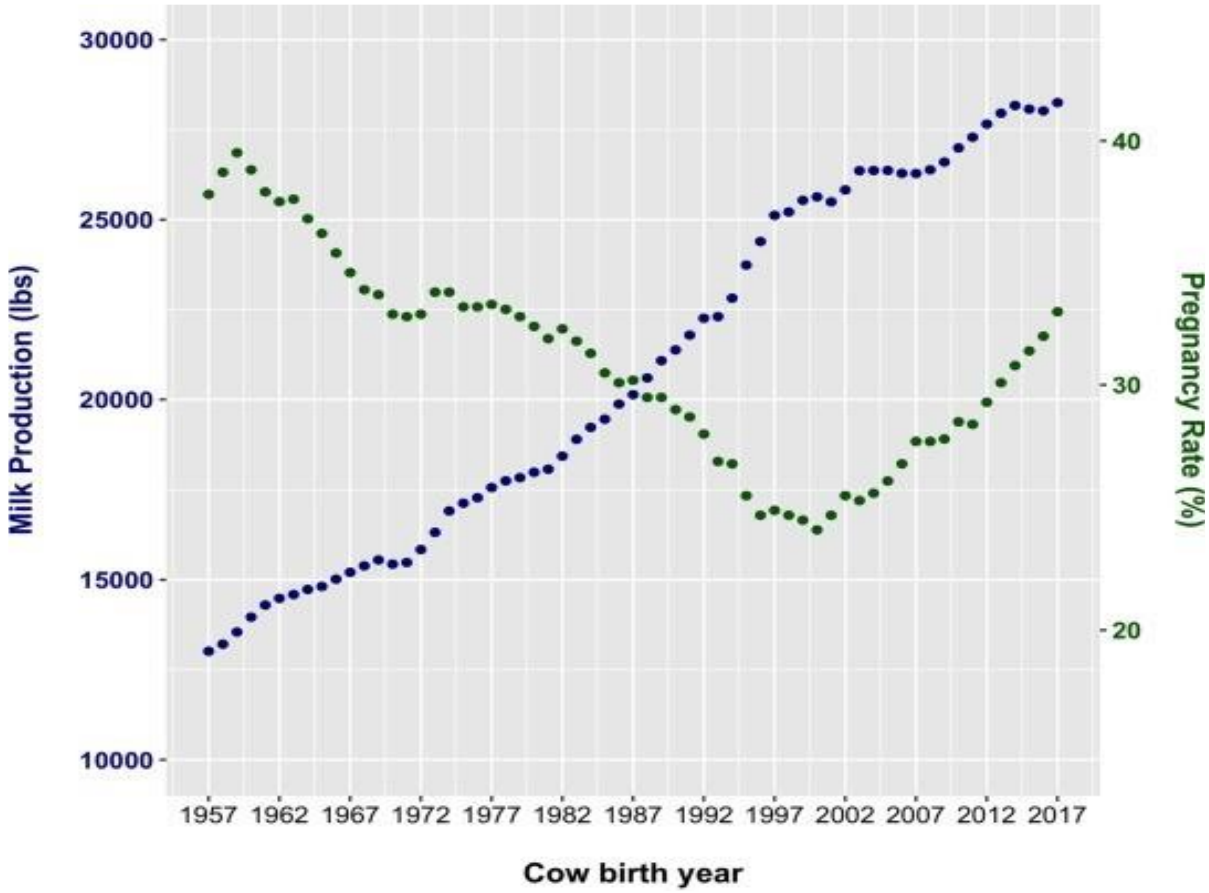


Fig 1.1: Changes in milk production (lbs) and pregnancy rate (%) of US Holstein cattle over six decades (Brito et al. 2021) data source: Council on Dairy Cattle Breeding website (July 2020; www.uscdcb.com)

In 2021 Brito et al. made a direct comparison between MY and PR over a 60-year period (Fig 1.1) clearly showing the negative genetic correlation between MY and fertility (Lucy, 2001; Pryce et al. 2004) until 2002, when inclusion of fertility traits in selection indexes has shown an improvement in PR that continue to be lower than those of the late 1960s (Brito et al. 2021).

Lower PR reduces LPL, while increasing culling and GHG emissions associated with rearing of replacement animals (Zehetmeier et al. 2014; Bell et al. 2015 Flysjö et al. 2011; Wolf et al. 2017) and lowering margins from milk production (Horn et al. 2012; De Vries, 2017). This led to fertility traits being included in genetic evaluation and selection index programs (VanRaden et al. 2004; Huang et al. 2007) and advent of genomic selection that has shown a steady improvement in genetic gain for PR (García-Ruiz et al. 2016). Improvements in the reproductive performance of dairy cattle continues to be a major priority (Roelofs et al. 2010; Fricke et al. 2014; Brito et al. 2021) and a major management criterion for the dairy industry. Closer inspection shows greater MY has been associated with a longer PPI to ovarian cyclicity and inconsistent oestrous cycle lengths (Lucy, 2003; Friggens and Chagunda, 2005), especially when MY accelerates quickly following parturition, elucidated considerable metabolic and physiological stressors (Domecq et al. 1997; Ingvarsten et al. 2003; Hansen et al. 2005) extending the PPI to conception, compared with a relatively flat and more steeply ascending lactation curve that reach peak MY and resumed pp ovarian activity sooner (Andersen et al. 2011). In addition to this lower CRs to first insemination < 60 d pp (Inchaisiri et al. 2010) occurs prior to peak lactation for cows that have higher MY, which in practical terms, led to a delay in first insemination from > 50 d pp to > 60 d pp for higher MY cows (Andersen et al. 2011) and a longer CI.

A greater PPI to the resumption of luteal activity and first oestrus has been found in cows with higher genetic merit for MY (Pollot and Coffey, 2008). This was related to the association between MY and regulation of fertility through the somatotrophic axis (Leroy et al. 2010a) related hormones consisting of insulin-like growth factors (**IGF -I and -II**) and growth hormone (**GH**), along with GH and IGF binding proteins (**BPs**) 1 to 6, and GH receptors (Garcia-Garcia, 2012). Both IGF and GH exert stimulatory, synergistic, or permissive effects on the hypothalamus pituitary gonadotropin (**HPG**) axis, reproductive tract and mammary gland (Chandrashekar et al. 2004) that affect reproduction, lactation and growth (Lucy, 2012) linked to metabolic status. The actions of IGF-I operate at multiple levels on the HPG axis coordinating reproduction and exciting the gonadotropin-releasing hormone (**GnRH)** neurons (Daftary and Gore, 2005) affecting the activity of the pituitary (Adam et al. 1998) and gonadotropin concentrations (Erickson et al. 1989) linking the reproductive and somatotropic neuroendocrine systems (Daftary and Gore, 2005). In terms of reproduction, the localised production of IGF-I plays a major role in controlling somatic cell function within the ovary, independent of GH, IGF-I and IGF IGFBPs, which have been involved in differing stages of follicular development and steroidogenesis (Demeestere et al. 2004, Webb et al. 2004), oocyte maturation (Lorenzo et al. 1996) and ovulation (Mazerbourg et al. 2003). Greater insulin, IGF-1 and leptin concentrations stimulate steroidogenesis and follicular development (Webb et al.

1999; Boelhauve et al. 2005; Walsh et al. 2011), along with greater concentrations of IGF-1 and luteinizing hormone (**LH**) to enhance follicular development (Lucy, 2000).

1.3: Dystocia, uterine disease, and fertility

Metritis and endometritis have been major health issues that contribute to suboptimal fertility due to the failure to resolve uterine inflammation, lack of interaction between GH and IGF-1, severe loss of body condition score (**BCS**) and failure to resume pp cyclicity due to low plasma LH, oestradiol and IGF-1 concentrations (Walsh et al. 2011) (Fig 1.2). The excessive allocation of glucose to the mammary gland contributes considerably to peripartal immune dysfunction (Habel et al. 2020) and infertility of dairy cows due to metritis, endometritis, pyometra, retained foetal membrane (RFM) and non-specific infections of the uterus (Walsh et al. 2011; Deori and Arundhati, 2015; Negasee, 2020). While pp pathogenic microbial uterine infections make metritis and endometritis more common, increasing infertility (Iain and Sian, 2017). Metritis is characterized by an enlarged uterus containing a watery red-brown fluid to viscous off-white discharge that may have an unpleasant odour (Abdel et al. 2016), which occurs mostly within 10 to 14 d pp (Arundhat et al. 2015; Deori and Arundhati, 2015). While endometritis is an inflammation of the endometrium, uterus and mucus membranes (Abdullah et al. 2015) characterized by an infected discharge from the vulva appearing approx. > 21 d pp (Turk et al. 2011; Negasee, 2020) or mucopurulent discharge detectable in the vagina at 26 d pp (Abdel et al. 2016). Subclinical endometritis favours bacterial growth and the development of metritis and endometritis (MacKay and Miller, 2003; Mounir et al. 2017; Negasee, 2020). A BCAP greater than recommended increases the risk of dystocia and incidence of metritis and endometritis due to calving trauma and assistance increasing the risk of bacterial contamination (Chaffaux et al. 1991; Mounir et al. 2017; Negasee, 2020).

Metabolic disorders, such as hypocalcaemia and ketosis, high BCSP and negative energy balance (**NEB**) increase the incidence of metritis, endometritis and retained placenta (Markusfeld, 1987), which has been more common in heifers of an AFC greater and lower than the 22 to 24 mo. recommended (Heinrichs, 1993; Hoffman, 1997; Gabler et al. 2000; Margerison and Downey, 2005; Cooke et al. 2013; Eastham et al. 2018). The occurrence, severity and persistence of uterine infections depend on the degree of bacterial contamination, uterine immune capability (Konigsson et al. 2002) and presence of RFM that provide substrates for microbial growth (Arundhat et al. 2015).

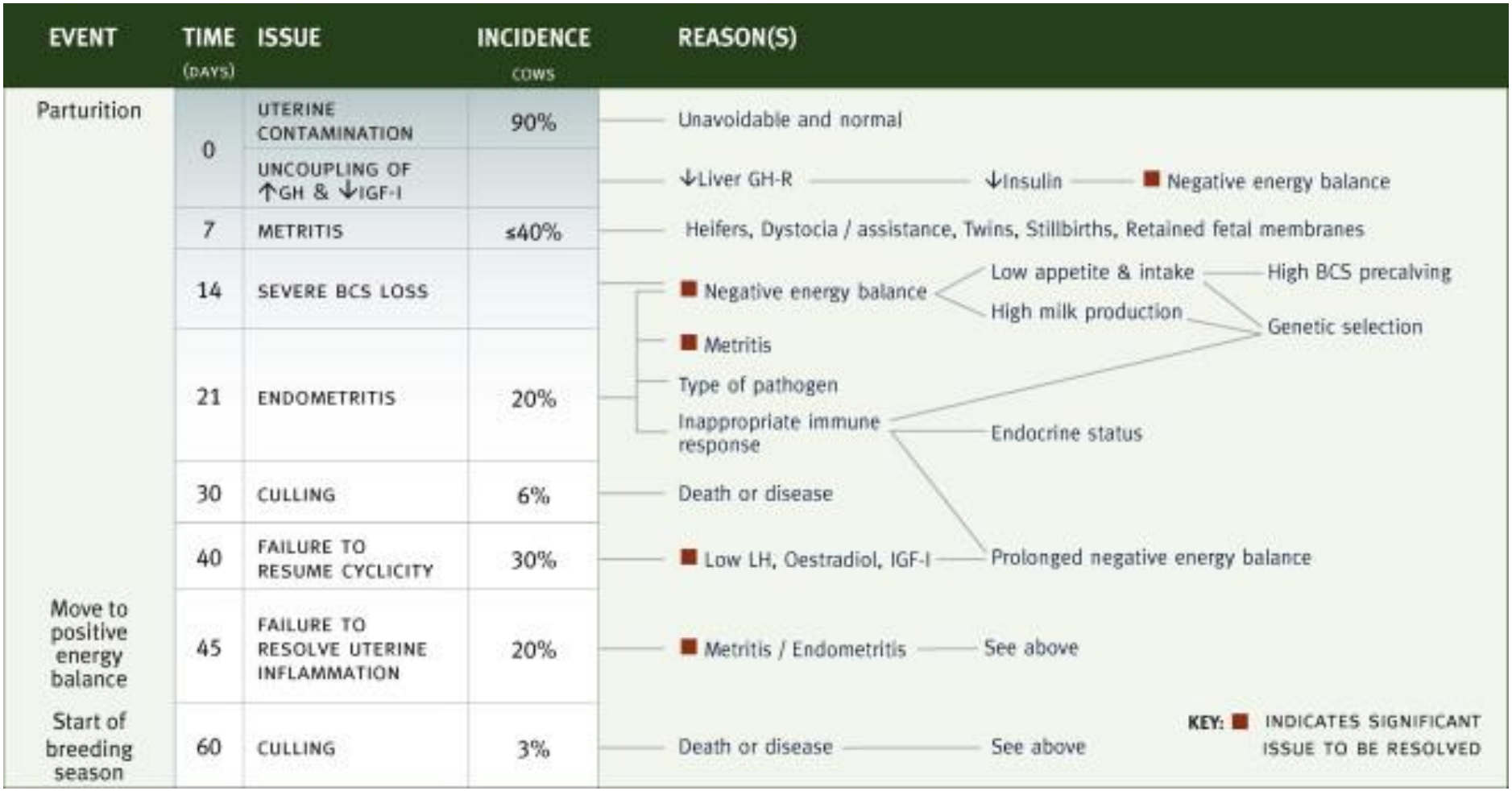


Fig. 1.2: Schematic presentation of the reasons for the major problems contributing to low fertility in dairy cows in the postpartum period (Walsh et al. 2011)

The associated inflammation reduces the rate of uterine involution, delaying the onset of ovarian activity and lowering CR, increasing the inseminations required, PPI to conception and cow morbidity, which lowers milk yield (Deori and Arundhati, 2015; Gautam et al. 2009; Negasee, 2020). Post-partum metritis was shown to increase the PPI to the first insemination by 7.2 d, reduce CR to first insemination by 20% and increasing the PPI to conception by 18.6 d (Youngquist and Shore, 1997; Smith et al. 2002; Walsh et al. 2011) (Fig 1.2). While clinical endometritis increased the PPI to the first insemination by 11 d, delay PPI to conception by 32 d and cows that had clinical endometritis later, between 20 and 33 d pp, were found to be 1.7 times more likely to be culled due to infertility (Youngquist and Shore, 1997; LeBlanc et al. 2014; Negasee, 2020). Clinical metritis and endometritis cause economic losses related to delay in the resumption of ovarian activity, increased number of services per conception, decreased milk yield, increased treatment costs and cause mortality due to septicaemia (Kasimanickam et al. 2014), making them some of the most disruptive diseases in terms of dairy cow reproductive performance and profitability (Mounir et al. 2017).

1.4: Postpartum resumption of ovarian function and ovarian cyclicity

One of the most crucial processes has been the PPI to uterine involution and reestablishment of ovarian cycles (Garverick and Smith, 2016). Normally uterine involution involves a decrease in the size of the uterine, degeneration and removal of fluids, which has been followed by the immediate rejuvenation and epithelialization of the mucosa of the uterus (Thatcher et al. 1984). A variety of bacteria have been commonly established in lochia, but have been completely absent in some uteri by 60 d pp (Roberts, 1971) and early reports indicate that the cervix opens rapidly and decreases in thickness (Morrow et al. 1969) shortly after parturition. Fonseca et al. (2010) reported that follicular growth begins rapidly following parturition, while ovulation has been delayed pending adequate LH, pulse frequency and amplitude, to generate ovulation of the dominant follicle(s).

The physiology of the ovarian cycles was reviewed by Roberts (1971) and typical ovarian cycles have been between 18 and 24 d in length (Gaines et al. 1993), which includes ovulation, which takes approx. 28 h following oestrus and lasts approx. 10 to 12 h (Morton, 2004) in dairy cows. This has been marked with abrupt increase in LH produced from the pituitary gland, when the oocyte finds its way into the oviduct following ovulation and the CL has been formed (Morton, 2001) inaugurating the intervention of oxytocin and progesterone (P_4) (Lucy et al. 1992). Progesterone concentrations continue to the greater until approx. 16 d following ovulation and then declines when the CL relapses (Morton, 2001), which has been induced by prostaglandin F2α produced by the inner layer of the uterine wall. Ultrasonic evaluation of ovarian anatomy has shown that one or two follicles grow and then degenerate throughout the

cycle prior to ovulation (Morton, 2004) and short cycles occur in most (70%), but not all cows following first ovulation (Crowe and Mullen, 2013) (Fig 1.3). Delays in PPI to the onset of ovarian cyclicity and first oestrus have been noted amongst dairy cows mainly consuming pasture in seasonal calving herds and AYR calving herds that have been offered silage and concentrate diets (Verkerk et al. 2000; Fonseca et al. 2010; Westwood et al. 2010).

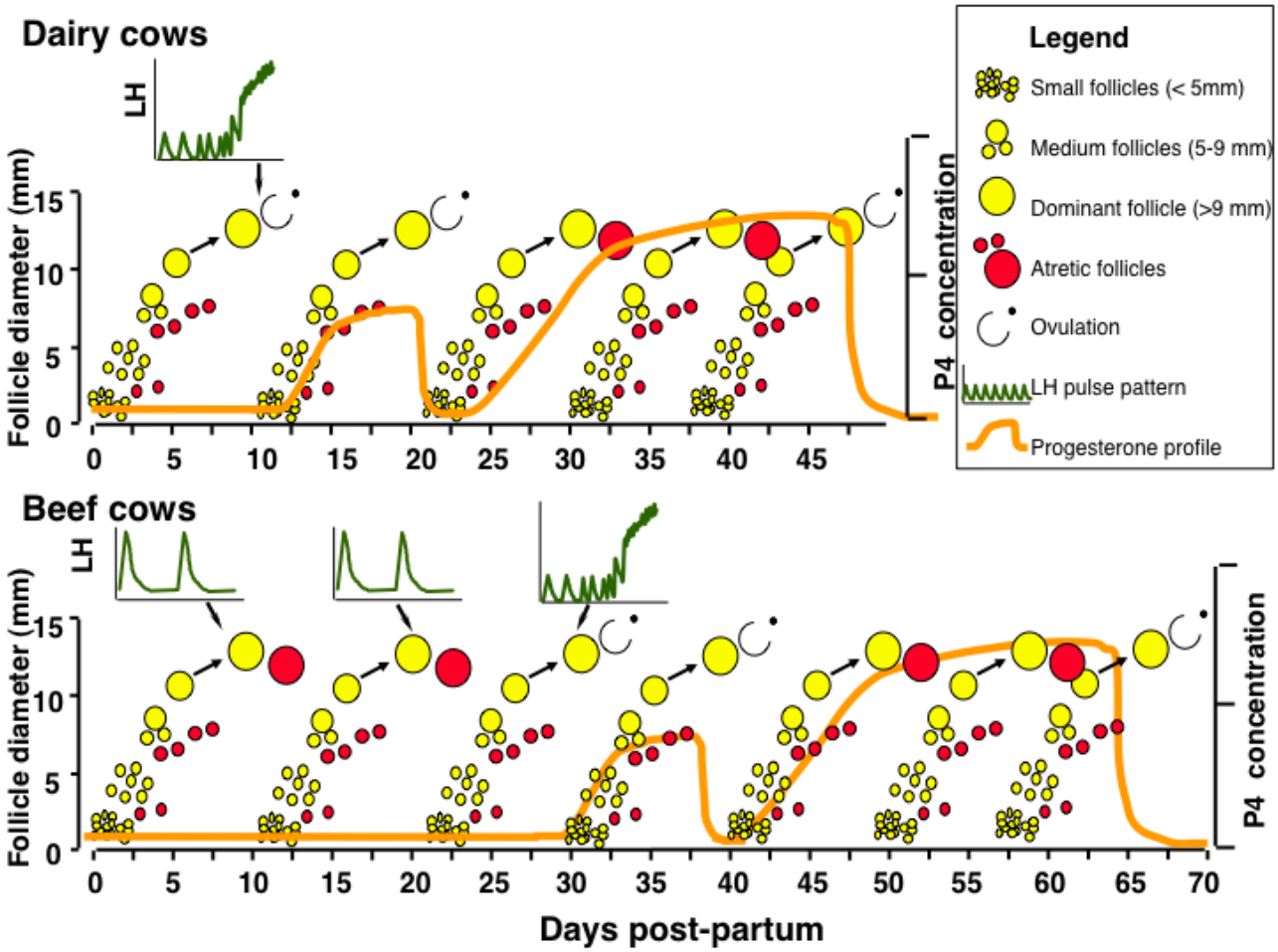


Fig 1.3: Resumption of dominant follicles and ovarian cycles during the postpartum period in dairy and beef suckler cows not nutritionally stressed (Crowe and Mullen, 2013, from Crowe, 2008)

The ovary responds directly to metabolic (Chagas et al. 2007) and folliculogenesis has been one of the more important events modulated by energy balance (**EB**) (Garcia-Garcia, 2012) and ovarian follicles play a major role in the oestrous cycle, oestrous behaviour, oocyte competency and subsequent embryo survival rate, and post-ovulation CL function and progesterone synthesis (Diskin et al. 2003). While gonadal function has been regulated by pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and these have been all modulated by nutrition (Webb et al. 2004; Garcia-Garcia, 2012). Energy,

especially glucose followed by fatty acid oxidation stimulate folliculogenesis (Garcia-Garcia, 2012), while proteins, vitamins, and other micronutrients play permissive rather than regulatory role (Scaramuzzi et al. 2010). This process has been modulated through metabolic response to a complex interaction (Scaramuzzi et al. 2006) between insulin-glucose, leptin and IGF I and II and IGFBP (Williams et al. 2001; Muñoz-Gutiérrez et al. 2004; Hunter et al. 2004). These are important mediators in the effect of dietary nutrient intake and/or EB (Diskin et al. 2003) and direct effect of nutrition on ovary function depends on IGF-I from liver and IGFBP concentrations in addition to a reduction in follicular responsiveness to LH, which has been initiated by insulin suppression of follicular oestradiol production to below the preovulatory-GnRH surge (Schneider, 2004; Diskin et al. 2003).

1.5: Monitoring ovarian cycles and fertility using milk progesterone concentrations

A cycle characterized by the recurrent appearance of oestrus or 'heat' at approx. of 21 d indicates regular ovulation (Pushpakumara et al. 2002) and concomitant increase in oestrus behaviour / activity, which has been used in commercial practice to ensure dairy cows have been served following ovulation (Roelofs et al. 2010) and potential conception. There have been many factors that affect the success of this process, especially in high-yield dairy cows. High ambient temperatures adversely affecting the potential success of fertilization (Rutledge, Monson, Northey and Leibfried-Rutledge, 1999) lowering CR (55.3%) (Sartori et al. 2002) and fertility of dairy cattle throughout the summer season in tropical, subtropical and temperate regions (Kastelic and Ginther, 1991; Roth et al. 2001). This has been likely to be due to decreased duration and intensity of oestrus behaviour, which interferes with the accurate detection of ovulation, especially in higher milk yielding dairy cows that has been exasperated during periods of heat stress (Walsh et al. 2011).

Equally poor CR can be due to lower oocyte quality, which has been related to modifications in the biochemical composition of follicular fluid lowering glucose, IGF-1 and cholesterol, along with higher plasma non-esterified free fatty acids (**NEFA**) and urea concentrations (Edwards et al. 1997) associated with lower feed intake and mobilization BCS. Other environmental factors include overall nutrient availability, feeding, housing, season and herd / group size and number of cows in oestrus at any one time (Kastelic and Ginther, 1991). The regular evaluation of milk progesterone (**P_4**) concentrations have been used to monitor ovarian function following parturition (Mann et al. 2005), to successfully identify reproductive cycle related issues, such as the resumption of PPI luteal activity, inter-ovulatory intervals, PPI luteal and intergluteal intervals (Opsomer et al. 1998; Royal et al. 2000; Sauer et al. 2004), pregnancy and calculate PR (Bulman and Lamming, 1979) but has limited effect on PR, potentially due to variation in P4 concentrations and small number of animals involved (Adriaens et al. 2018). Circulating

maternal progesterone concentrations being the main hormone associated with the uterine environment and early embryonic development (Adriaens et al. 2017) (Fig 1.3). Milk P_4 concentrations can be used to identify long pp even in the absence of oestrus, with or without pathological symptoms such as vaginal discharge and the recurrent abnormal oestrous activity commonly associated with cystic follicles (Lamming and Darwash, 1998; Royal et al. 2000), persistence of the carpus luteum (**CL**) and late embryo mortality (Lamming and Darwash, 1998). The application of regular monitoring of milk P_4 concentrations has been applied to evaluate potential causes of poor reproductive performance and synchronization of ovulation and artificial insemination (**AI**), which can improve reproductive management and fertility of dairy cows (Bruinjé et al. 2019). The historical evaluation of P_4 concentrations, prior to and following insemination, can be used to calculate reproductive efficiency and the economic implications of infertility on productivity including CI and MY (Lamming and Darwash, 1998).

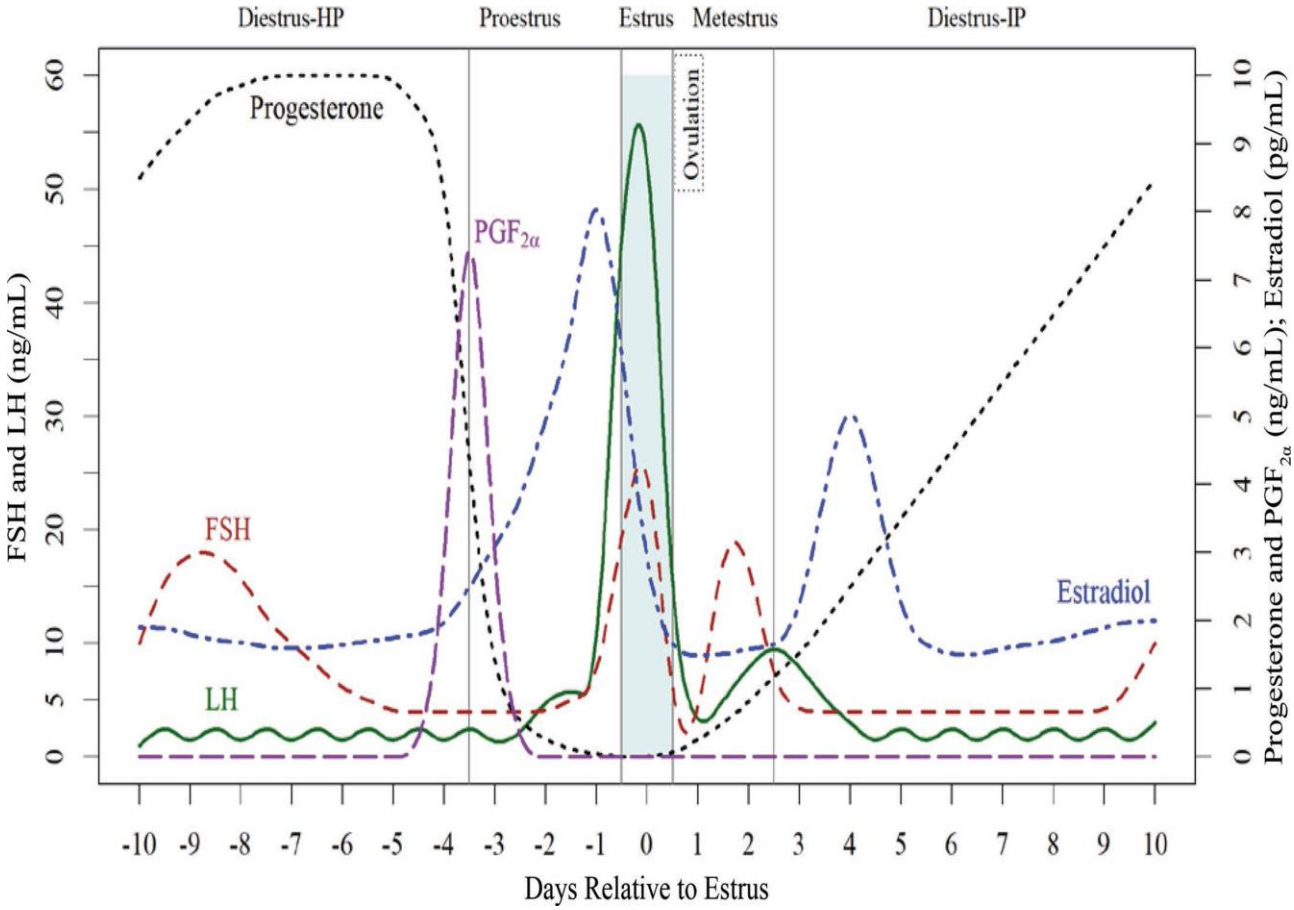


Fig. 1.4: Schematic depiction of the pattern of secretion of progesterone, FSH, LH, $PGF_{2\alpha}$, and oestradiol (estradiol) during the oestrous cycle in cattle (Toledo-Alvarado **et al. 2018)** (Dioestrus (Diestrus): High-progesterone (diestrus-HP) ranging from −10 to −4 d; Proestrus: ranging from −3 to −1 d; Oestrus at d 0; Metoestrus from 1 to 2 d; Dioestrus: increasing-progesterone (dioestrus-IP) 3 to 10)

The incidence of cycle related issues was found to be low, the animals affected showed significantly impaired reproductive function (Mann et al. 2005) that has considerable potential impact on culling and replacement rates. Involuntary culling due to infertility, related to failure to conceive and other fertility-related issues, has been one of the most common causes of removal of cows from dairy herds, followed by mastitis / high somatic cell count and lameness (Gardner et al. 1990; Miller and Dorn, 1990, Forbes 2000). These forced culling events upto the ≈ 20% may leave little opportunity to select other production-related traits and improve herd genetics without increasing the economic and environmental cost of herd replacement (Margerison, 2022). Unfortunately, CI has progressively increased (Hare et al. 2006) and average UK CI for Holstein–Friesian cows has been reported at 428 d (75th quartile 463 and 90th decile 546 d) (Gates 2013) and culling of pregnant animals increases when herds have longer CIs (Parkinson, 2019). A regular annual 365 d CI is especially important for regular seasonal calving, while greater CI may be more appropriate (375 d) when greater individual cow MY (>9,000 l/cow/year) (Margerison, 2022). However, greater CI, AFC and infertility related culling all increase the number of replacement livestock required to produce the same amount of MY, which has been detrimental to economic and environmental sustainability of dairy production (Margerison, 2022).

Typical ovarian activity occurs in approx. 60% of dairy cows, while atypical cycles have been found to be common in the remaining 40% of the dairy cow population, which typically includes delayed commencement of luteal activity (Petersson et al. 2006; Windig et al. 2008; Cutullic et al. 2011). While between 13 to 30% of the variation in ovarian cyclicity has been related to genetics and genetically negatively correlated with MY (Veerkamp et al. 2000; Royal et al. 2002b; Petersson et al. 2007). Holstein Friesians show more abnormal cyclicity compared with some other breeds, such as Jersey, especially in terms of delayed PPI to first ovulation (Petersson et al. 2006; Cutullic et al. 2011; Piccand et al. 2013), however, the deleterious effect of high genetic merit for MY on cyclicity is complex (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and has been related to body condition score, health and nutrition.

1.6: Monitoring oestrus activity

The basic physiology of the ovarian cycles and oestrus were reviewed by Roberts (1971) and oestrus was defined as the period within the ovarian cycle when the cow has been sexually receptive, which takes place over a relatively short period of 7 (Hurnik, King and Robertson, 1975) to 12 h period (Morton, 2004; Mária et al. 2018; Parkinson, 2019) while some studies found no effect of MY on oestrus behaviour (Lyimo et al. 2000; Van Eerdenburg et al. 2002) some studies show a negative effect of greater MY on activity (López-Gatius, 2005), standing

to be mounted and total time standing (López et al. 2004; Rivera et al. 2010) and variation in the total number of mounts per oestrus has been noted to exist between individual cows (Xu et al. 2010). Using activity monitoring technology, it has been shown that the start of oestrus-related behaviour was 6 h before and 3 h following the start of an activity cluster recorded by Heatime® (SCR Engineers Ltd, Netanya, Israel) activity monitors (Aungier et al. 2015). This acceleration technology can be attached to the neck collar or in an ear tag of individual cows (Madureira et al. 2015; Gaillard et al. 2016), which continuously measures horizontal acceleration that has been related to upward movements of head and neck during walking and mounting behaviour (Reith et al. 2014). This data stored at 1 and 2 h intervals (Reith et al. 2014; Madureira et al. 2015; Gaillard et al. 2016) and used to calculate a daily activity index (Silper et al. 2015). These are interpreted, using algorithms based on deviations from typical activity patterns, to separate days that have increased activity associated with oestrous behaviour, which has been automatically transferred via an antenna to herd management software to provide graphs and lists that facilitate the identification of the reproductive, rumination and health status of individual cows (Reith et al. 2014). The percentage of correctly alerted oestrus using accelerometer systems range between 71 (Valenza et al. 2012) and 90% (Aungier et al. 2015), while detection rates did not differ between accelerometer systems and heat mount detectors (71% v. 66%, respectively), verifying accelerometer systems have been a useful tool in the detection of oestrus and improvement of dairy cow fertility (Valenza et al. 2012) by achieving a more effective alignment between oestrous and insemination because the longer semen dwells within the oviduct prior to ovulation lowers the chances of fertilization (Dalton et al. 2001).

1.7: Conception and establishment, recognition, and maintenance of pregnancy

In dairy cows, approx. 30 h following the onset of oestrus (Robinson 1979, Roelofs et al. 2005) fertilization occurs while the oocyte travels approx. one third of the way down the uterus, which has remained remarkably constant despite reductions in oestrus behaviour and prolonged interval between the onset of oestrus and ovulation (Bloch et al. 2006). The highest CR rates occur when insemination has been completed from the middle to the end of standing oestrus, i.e., 8 to 13 h prior to ovulation (Saacke et al. 2000, Nebel et al. 2000; Parkinson, 2019), which in practice equates to cows that have been observed in oestrus in the morning being submitted for AI in the afternoon and cows observed in oestrus during the afternoon being submitted for AI the following morning (Parkinson, 2019), while Nebel et al. (2000) suggested that, if the time of onset of oestrus has been not precisely known, insemination should take place within 6 hours of the initial observation of oestrus. Gordon (1999) reported that fertilization occurs in the oviduct following the deposition of sperm in the uterus, requiring 6 to 8 h for an adequate population of semen to reach the oviduct and result in potential fertilization (Nebel, Dransfield,

Jobst and Bame, 2000) and followed fertilization the conceptus (Hubbert, 1972) travels down the oviduct and arrives at the uterus 72 to 84 h later (Gordon, 1999).

The CL has been retained by the existence of the conceptus and without implantation the CL will persist for approx. 16 d due to prostaglandin F2α excretion from the endometrium (Morton, 2001; Spencer et al. 2013; Arosh et al. 2015) and Thatcher et al. (1984) show that this was critical for the maintenance of pregnancy, which occurs, at least in part, this was due to biochemical signals from the conceptus (Arosh et al. 2015; Sanchez et al 2018). In the ruminants, maternal recognition of pregnancy has been mediated by conceptus-derived trophoblastic interferon-τ (IFNτ) (Fig 1.5), which inhibits the release of oxytocin that has been driven luteolytic pulses of prostaglandin PGF_2alpha (**$PGF_{2\alpha}$**) from the epithelial cells of the endometrium and stimulates the production of prostaglandin E_2 (**PGE_2**) (dinoprostone) from the stromal cells that has luteo-protective effects (Antoniazzi et al. 2013; Kumawat et al. 2022). Moreover, intra-luteal prostaglandin biosynthesis and signalling have been selectively directed towards PGE_2 during the establishment of pregnancy, but towards $PGF_{2\alpha}$ during luteolysis (Lee et al. 2012).

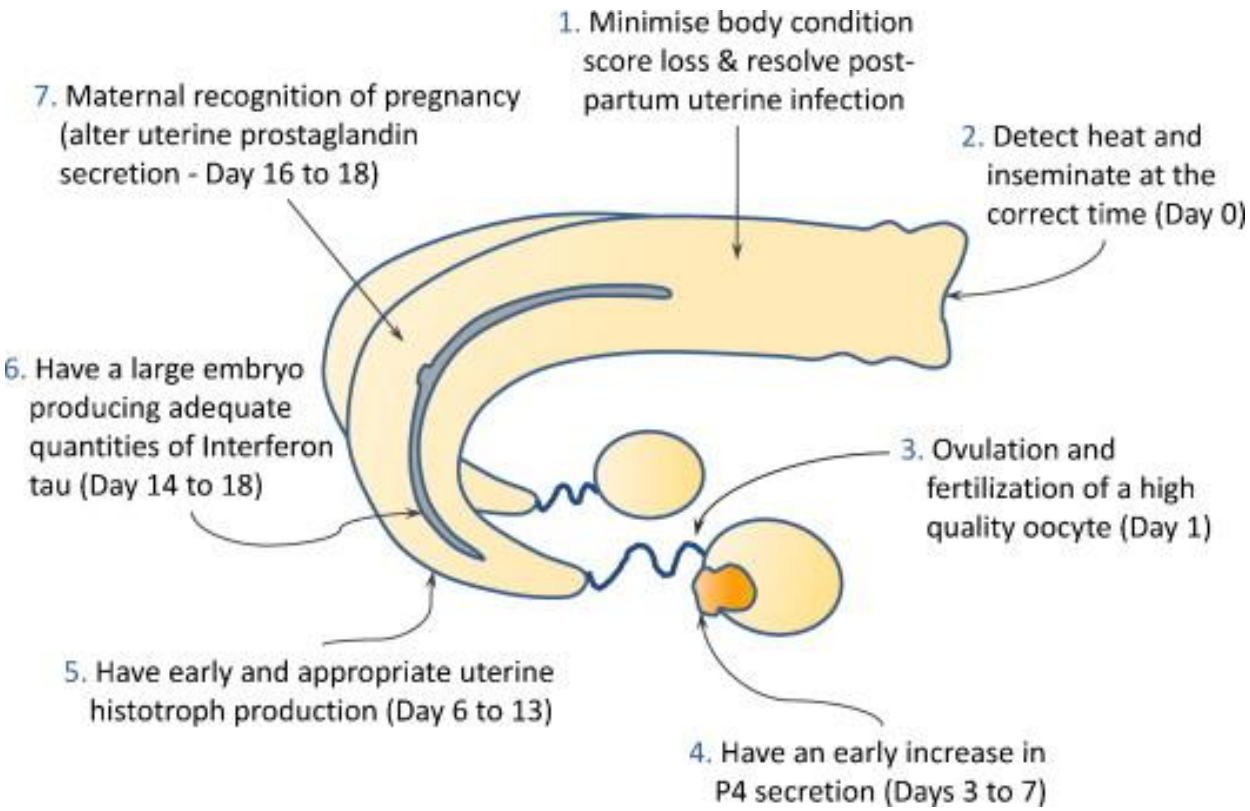


Fig. 1.5: Schematic presentation of the most important factors affecting the establishment of pregnancy in dairy cows (Walsh et al. 2011)

The attachment of the placenta to the uterine wall begins at approx. 20 d following conception (Vanroose, de Kruif and Van Soom, 2000), followed by elongation (Dorniak et al. 2011) while the growth rate of the foetus (> 45 d, Hubbert, 1972) has been most prolific up until 240 d in dairy cows and this then slows and parturition occurs at approx. 282 d of gestation (Nadarajah et al. 1989; Garverick and Smith, 2016) unless high MY prolongs gestation (Silva et al. 1992) or gestation has been shortened by sire selection (248 to 279 d) (Nogalski et al. 2012) or higher summer temperatures (McClintock et al. 2003). The conceptus can be lost prior to parturition and considerable damage can occur within 16 to 18 d following implantation in beef and lactating dairy cows (Roche, Bolandl and McGeady, 1981; Diskin and Morris, 2008) while loss of the foetus at >56 d of pregnancy has been less common (Forar, Gay and Hancock, 1995), however, both longer and shorter gestation periods contribute to a higher number of stillbirths (Norman et al. 2009).

An inadequate supply of nutrients, specifically but not limited to energy, can restrict foetal growth due to an adverse intrauterine environment, but preserves the development of vital organs, despite an overall reduction foetal body size and weight in pigs (Pond et al. 1992), rats (Simmons et al. 1993). While the majority of research was completed in mid- and late-gestation (Hough et al. 1990; McGee et al. 2006; Taylor et al. 2016) and the umbilical cord diameter and crown-rump length in relation to thoracic diameter of beef cattle were affected as early as 39 d of gestation, which followed by compensatory / preferential foetal growth in response to greater nutrient availability (Gonzalez et al. 2013; Micke et al. 2010). Undernutrition, overnutrition and supplementation of the dam affects the development of the components of the HPG within the offspring (Rhind, 2004), including the sensitivity of GnRH and Kiss1r mRNA expression that has been established during the early neonatal period (Iwasa et al. 2010). Maternal nutrition has been found to have no effect on the MY, BCS and fertility of offspring during the first lactation (Banos et al. 2007), which indicates the biological importance of nutrition and metabolic status, which has a greater direct effect on MY of the dam than longer-term effect of maternal nutrition on the MY of the offspring (Bach, 2011).

1.8: Body condition score at parturition and fertility

Reproductive abnormalities have been more common in dairy cows that have been above target BCSP (>3.0), which has been potentially mediated by pituitary insulin signalling (Brothers et al. 2010; Sagae et al. 2012) affecting oocyte development (Purcell and Moley, 2011; Yang et al. 2012). While greater body fat increases leptin concentrations, which act on the hypothalamus stimulating GnRH and gonadotropin secretion (Yu et al. 1997; McCann et al. 1998) directly inhibiting ovarian steroidogenesis (Spicer, 2001; Kendall et al. 2004) leading to a decrease in oestradiol secretion (Spicer and Francisco, 1997). Resistin, an adipokine,

expressed in bovine ovaries can inhibit steroidogenesis and follicular development (Spicer et al. 2011) by modulating granulose cells functions in the basal state or in response to IGF-I *in vitro* (Maillard et al. (2011) and adiponectins reduce GnRH secretion (Cheng et al. 2011) and inhibit GH and LH release (Rodriguez-Pacheco et al. (2007) that stimulate steroidogenesis by granulosa cells in the ovary (Monget et al. 2008). Plasma leptin concentrations have been increased by body fat deposits, lowering oocyte maturation (Craig et al. 2004), follicle rupture and corpus luteum formation (Ruiz-Cortés et al. 2003), embryo implantation and establishment of pregnancy (Craig et al. 2004).

The EB has been not directly measurable on farm, and it has been monitored through BCS (Roche et al. 2009; Friggens et al. 2010), which gives a good indication of body fat reserves (Domecq et al. 1997; Garnsworthy, 2006; Roche et al. 2009; Berry et al. 2021) and labile protein. Energy balance can be estimated based on body weight and BCS (NRC, 2001; Banos, et al. 2006) or based on the differences between energy intake and energy requirements for maintenance, milk production and fertility (Patton et al. 2007; Aguilar-Perez et al. 2009), however BCS has been used as a proxy measure that has been a quick on farm assessment that can be completed regularly and applied to specific groups of cows at differing stages of production and/or compared with target BCS (Table 1.2), to estimate EB and NEB by physiological change in BCS. This approach was first proposed by Lowman et al. in 1973, adapting a BCS system for beef cattle and the effect of BCS parturition on MY, reproduction, health and welfare and 5 and 10-point scales have been applied (Edmonson et al. 1989; Garnsworthy, 2006; Roche et al. 2009). In the UK a 5-point (1: Thin; 5: Fat) scale that has recently been refined from 0.5 to 0.25 increments (Ishler et al. 2016) (AHDB, 2020) can be compared with recommended BCS targets for dairy cows at partition, 60 d pp, 100 d prior to and at drying off (Table 1.2) to manage the nutrition, fertility, health and MY of dairy cows.

Table 1.2: Recommended body condition score and change in body condition score for dairy cows in Great Britain

Stage of lactation	Target BCS	Stage of lactation	Max change in BCS
Parturition	2.5 to 3.0	0 to 60 d pp	- 0 to 0.5
60 d post-partum	2.0 to 2.5	60 to 100 d pp	+ 0 to 0.5
100 d prior to drying off	2.5 to 3.0	Late lactation	+ 0 to 0.5
At drying off	2.5 to 3.0	Dry period	0

Amended from AHDB, 2020 and 22 https://ahdb.org.uk/knowledge-library/body-condition-scoring-flow-chart

The BCS of dairy cows at partition has been managed in late lactation or dry period of cows dried off before 305 d pp (Margerison 2022) and during the final three weeks prepartum, 'transition cows' have been offered diets that meet energy requirements for maintenance of the cow and foetal growth (Grummer, 2007) avoiding BCS gain. Dairy cow nutrition during the transition period, from appro. 3 prior to and 3 weeks pp, has been characterized by a period when dairy cattle respond to the metabolic challenges of late pregnancy and early lactation (Bell, 1995) and good nutritional strategies and cow management during this period can lower metabolic disorders, improve pp DMI, MY, EB and fertility (De Veth et al. 2009) and PR of dairy cows by <30 % (DeGaris et al. 2010 a, b). Dairy cows that have a higher than recommended BCS at parturition (3.5) have a greater risk of retained placenta and/or metritis (Markusfeld et al. 1997), twice as likely to develop ketosis (Reid et al. 1986) and at 2 to 4 times more likely to suffer ketosis in the following lactation (Rasmussen et al. 1999) and metabolic disorders (Acosta-Martinez, 2011). Moreover, leptin is a 16 kD protein, which is synthesized primarily by adipose tissue (Garcia-Garcia, 2012) and it modulates a range of biological functions, including reproduction and energy homeostasis (Barb et al. 1999; Clarke and Henry, 1999).

The amount of body fat directly affects plasma concentrations of leptin (McGregor et al. 1996), which has a potent effect on satiety (Campfield et al. 1995) and considerable feed intake, via the neuroendocrine-axis (Cervero et al. 2006) and has been a key link between nutrition, metabolic status and reproduction (Clarke and Henry, 1999). Dairy cows that have been over conditioned at parturition have a greater frequency of abnormal cyclicity patterns (Cutullic et al. 2012; Bedere et al. 2016) and body condition per se and body condition loss influence commencement of luteal activity, while MY per se does not, however the occurrence of prolonged luteal phase may be related to MY (Royal et al. 2002b; Kafi et al. 2012) due to the biological effect of MY on BCS experimental outcomes maybe confounded by these factors. Finally, dairy cows that have a BCS at parturition (BCSP) lower than industry target (Table 1.2) suffer a greater incidence of metritis (Schroder and Staufenbiel, 2006; Daros et al. 2019) have longer PPIs to the commencement of ovarian activity (Roche et al. 2007), lower CR to first service (Lopez-Gatius et al. 2003; Bedere et al. 2018), longer PPI to conception (and CI) and lower PR (Schroder and Staufenbiel, 2006; Daros et al. 2019). Both low BCS at calving and/or high amounts of BCS mobilization during early lactation increase the risk of a delay in the commencement of luteal activity (Cutullic et al. 2012; Bedere et al. 2016).

1.9: Postpartum change in body condition score and fertility

Dairy cows mobilise BCS during early lactation to sustain MY (Pires et al. 2013; McCabe and Boerman, 2020) and BCS reserves at parturition affect subsequent MY and composition (Schroder and Staufenbiel, 2006; Pries et al, 2013) and peripartum changes in BCS directly affect the productivity, health, and reproductive performance of dairy cows (Garnsworthy, 2006; Souissi and Bouraoui, 2019). Dairy cows have been genetically predisposed to lose BCS pp and small amounts of BCS loss have been unlikely to have a dramatic effect on MY and fertility (Pryce et al. 2001; Friggens et al. 2004; Garnsworthy, 2006), however, higher yielding dairy cows, that have greater metabolic demands and those that have a BCSP above industry standard (3.0 to 3.5) (Markusfeld, Ezra and Galon, 1997) typically mobilize more BCS to support MY due to the greater limitations of pp DMI (Drackley and Reynolds, 2021; Margerison, 2022) and experience a greater NEB compared with cows in optimum BCSP (2.5 to 3.0) at parturition (Gwazdauskas, Wilcox and Thatcher, 2010) (Table 1.2). Dairy cows that have a BCS loss < 0.5 return to pp ovarian activity and first ovulation within 30 d of parturition, whereas cows that have greater pp BCS loss (> 0.5 BCS) during early lactation (< 70 d pp) have lower CR and longer CI (Pryce et al. 2001; Garnsworthy, 2006; Patton et al. 2007). Cows that lose > 0.5 to 1.0 points of BCS have PPIs to ovulation 6 d longer, while cows that had BCS loss > 1.0 points ovulated 20 d later at 50 d pp (Butler, 2005), when service may commence. Cows that lose > 0.5 BCS pp have lower CR (Collard et al. 2000; Pryce et al. 2001; Garnsworthy, 2006; Patton et al. 2007), which has been reduced by 10% per 0.5 loss in BCS pp (Butler, 2005) and a pp loss of >1.0 BCS extends the PPI to conception by 11 d compared with cows that lost 0 and 0.5 of BCS pp (Lopez-Gatius et al. 2003).

The effect of NEB and pp BCS loss > 0.5 on fertility has been related to lactational anoestrus delaying the onset of ovarian activity and oocyte quality, which lowers CR, and these have been mediated through endocrine regulation that changes lipogenesis to lipolysis during late gestation and early lactation and this reduces insulin secretion (Garcia-Carcia, 2012). These metabolic signals control EB link reproduction to plasma concentrations of insulin (Wallace, 1996) IGF-I (Rhind et al. 1998) and leptin (Mantzoros et al. 1996; Hardie et al. 1996) concentrations decrease when BCS decreases during periods of NEB due to periods of lower feed intake or when nutrient requirement outstrip nutrient intake and mobilization of BCS occurs (Garcia-Garcia, 2012), which occurs during early lactation of dairy cows and growing heifers suffering severe undernutrition, interfering with normal oestrous cycles and alter endocrine function (Short and Bellows, 1971; Houseknecht et al. 1988). The related plasma metabolite concentrations (Glucose, beta-hydroxy-butyrate (**BHB**), urea) modulate hormone input into the GnRH neurones and control GnRH release, which provides the appropriate stimulation of the ovaries (5) (Fig 1.6). In later lactation, post peak MY and/or when nutrient

intake increases when feed intake and nutritional status improves and BCS increases and concentrations of insulin (Wallace, 1996), IGF-I (Rhind et al. 1998), and leptin (Mantzoros et al. 1996; Hardie et al. 1996) decrease and this improves conception rate.

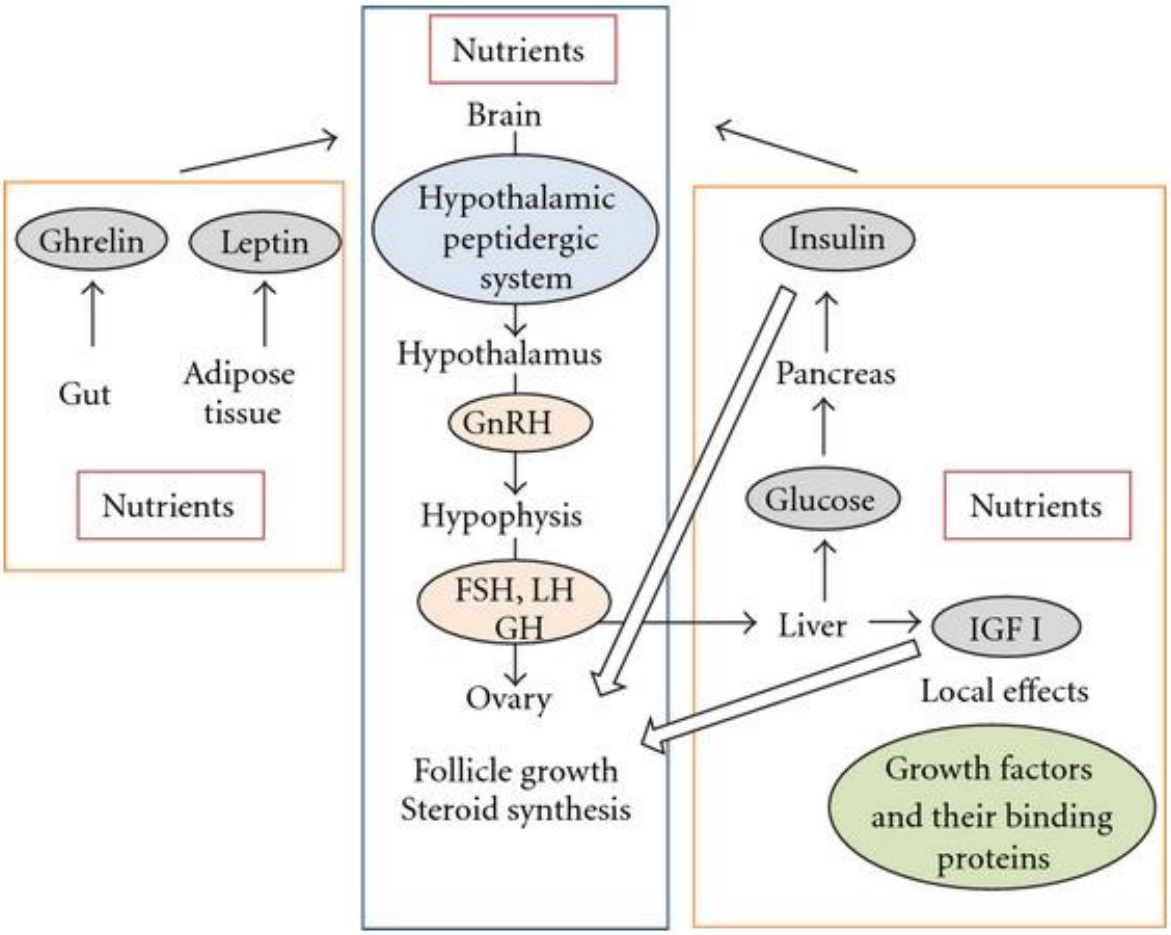


Fig. 1.6: Schematic representation of mechanisms through which nutrient intake and adipose tissue affect reproductive function (Garcia-Garcia, 2012)

Insulin and IGF-I may be mediators between BCS and ovarian follicle development, ovulation and embryo development (Chagas et al. 2007) and nutritionally induced changes in the ovarian IGF system play a key role in regulating oocyte quality (Nakae et al. 2001) and conception. Insulin has been a modulator of the metabolic stimulus, rather than a mediator between the level of internal energy and central effectors (Schneider, 2004). The common signalling pathway for IGF-I receptor and insulin receptors (IR) that operate via insulin receptor substrate (IRS) proteins (Nakae et al. 2003; Kim and Accili, 2002), which regulate fertility by directly affecting ovarian function (Scaramuzzi et al. 2006; Garnsworthy et al. 2008). The local production of IGF-I plays an important role in the intricate paracrine control of function of different types of somatic cells in the ovary independently of GH (Garcia-Garcia, 2012) and

IGF-1 and IGFBPs participate by means of various mechanisms in different stages of follicular development, follicular steroidogenesis (Demeestere et al. 2004, Webb et al. 2004) and oocyte maturation (Lorenzo et al. 1996) and ovulation (Mazerbourg et al. 2003). Normal concentrations of adipokines are fundamental in the maintenance of the integrity of HPG axis, regular ovulatory processes, successful embryo implantation and pregnancy (Tersigni et al 2011). Equally there are multiple levels of the HPG axis where IGF-I can coordinate reproduction, which is because IGF-I acts as a direct regulator of GnRH neuron, usually exciting the HPG axis (Daftary and Gore, 2005) via actions in the pituitary (Adam et al. 1998) and gonadal levels (Erickson et al. 1989). This indicates the potential link that IGF-I plays between the reproductive and somatotropic neuroendocrine systems (Daftary and Gore, 2005).

1.10: Postpartum and early lactation feed intake and fertility

During early lactation a lower than maximum feed intake provides an inadequate nutrient supply to meet the requirements for maintenance and all the production requirements of dairy cows (Friggens et al. 2010; Walsh et al. 2011; Margerison, 2022) inducing undernutrition, which can have a negative effect on ovarian follicle development (Alexander et al. 2007), ovulation (Yan et al. 2008), blastocyst formation (Borowczyk et al. 2006), and fertility rates (Evans and Anderson, 2012). Long (chronic) and short term (acute) undernutrition suppress gonadotrophin-releasing hormone (**GnRH**) secretion, inhibiting or delaying the preovulatory pulsatile peak release of LH and lowers plasma oestradiol-17β (Boland et al. 2001; Rhind et al. 1989), while increasing progesterone concentrations (Kiyma et al. 2004). This altered endocrine function interferes with the occurrence of typical / normal oestrous cycles (Houseknecht et al. 1998; Short and Bellows, 1999) (Fig 1.7).

Leptin concentrations have been equally sensitive to short-term alterations in feed intake and energy balance (**EB**) (Cunningham et al. 1999; Marie et al. 2001) and the ability to increase energy oxidation (Schneider, 2004), which affects glucose homeostasis and the action of insulin. Leptin impairs insulin sensitivity (Monget et al. 2008) and insulin stimulates the secretion of leptin by adipocytes and promotes lipogenesis (Poretsky et al. 1999), linking nutrition and reproduction through the influence of GnRH neuron activity (Tena-Sempere, 2007; Israel et al. 2012) and the promotion of GnRH function via an indirect action on kisspeptin neurons (Tena-Sempere et al. 2006; Quennell et al. 2011). Equally, serum ghrelin levels have been influenced by both short- and long-term changes in energy homeostasis (i.e., with glucose, insulin, and somatostatin levels) (Muccioli et al. 2011) and signal starvation or energy insufficiency and limiting reproductive function though a complex mode of action on

the gonadotropic axis, inhibiting hypothalamus GnRH-induced gonadotropin secretion and stimulation of basal LH and FSH secretion (García et al. 2007).

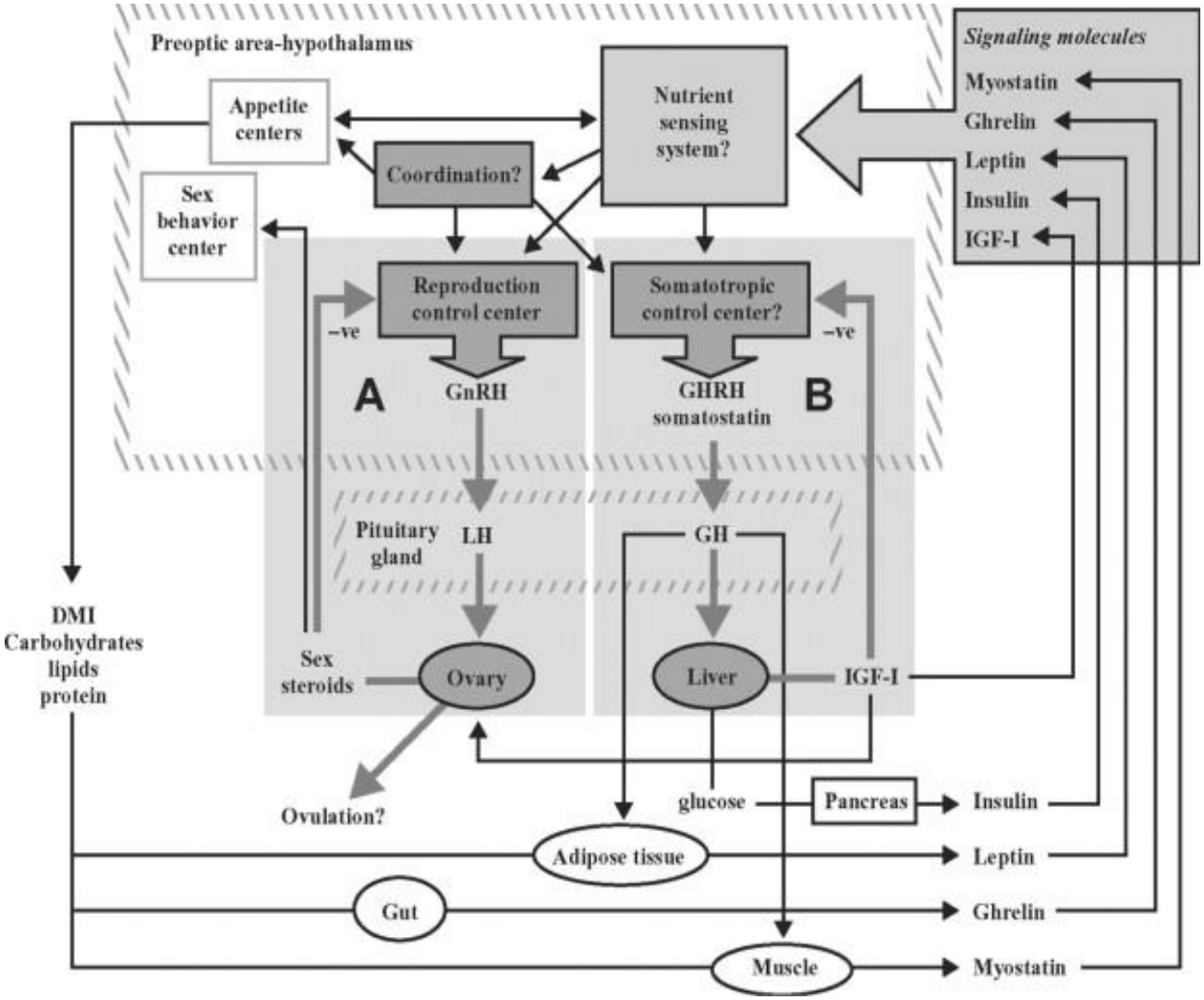


Fig. 1.7: Feedback-regulation systems that control the reproductive axis (shaded is A) and the somatotropic axis (shaded is B) interact at several levels and thus link nutritional and metabolic inputs into the reproductive process (Chegas et al. 2007) Note omissions and rational: Follicle stimulating hormone (FSH) from the reproductive axis, because it does not appear to limit dairy cow fertility; Thyroid and adrenocortical axes, because these have been regulated by their own feedback loops in the brain-pituitary system, intimately linked with lactation, respond to metabolic inputs, affect the reproductive and somatotropic systems, so they introduce inputs from stressors such as high ambient temperature, disease, and the antagonistic interactions associated with establishment of social dominance; Sex-steroid feedback, positive feedback for induction of the preovulatory surge)

The effect of NEB on MY and fertility have been demonstrated both genotypically and phenotypically (Veerkamp, 1998; Westwood et al. 2000; Pryce et al. 2004) and it occurs when

DMI cannot meet nutrient requirements for MY during early lactation (Mackey et al. 2007; Patton et al. 2007). Feed intake has been the primary factor eliciting the effect of EB on reproduction (Patton et al. 2007). Ghrelin has been involved in a wide spectrum of biological functions, including GH secretion and regulation of EB, and exhibits diverse array of effects that include glucose metabolism, secretions and motility of the gastrointestinal tract (Lorenzi et al. 2009), regulating energy homeostasis, which promotes DMI (Korbonits et al. 2004) and provides the central nervous system information regarding EB and maintenance of homeostasis (Hidekitamura et al. 2000; Nakazato et al. 2001; Hattori et al. 2001). Moreover, feed intake has been a principal factor that affects fertility (Webb et al. 2004; Robinson et al. 2006; Ashworth et al. 2009) amongst other factors, which include BCS (Roche et al. 2009), MY and milk composition (Friggens et al. 2007), metabolite and hormone concentrations (McDougall et al. 2005). Feed intake has been reported to account for approx. 50% of MY response to animal selection in addition to the mobilization of BCS to provide nutrients to support milk synthesis (Veerkamp, 1998) and reproduction. A recent study showed that variation in bovine myostatin genes between differing Holstein Friesian, Jersey and cross breeds increased the amount of unsaturated and lowering the saturated fatty acid concentrations in milk, but no assessment of its effect on fertility was undertaken (Haruna et al. 2020), however this may contribute to genetic differences in BCS mobilization.

1.11: Dietary and endogenous energy balance

At parturition the initiation of lactation and the increased demand for nutrients for corpus MY, combined with lower than maximum feed intake, providing inadequate amounts of nutrients for dairy cows to meet the requirements for maintenance of the body and production requirements (Friggens et al. 2010; Walsh et al. 2011; Mäntysaari et al. 2012, 2019; Margerison, 2022). The mobilisation of nutrients from body tissue due to NEB has been considered an adaptive mechanism that occurs naturally and managed effectively may not be affected fertility (Drackley et al. 2005; Friggens et al. 2010), however metabolism associated with the deposition and mobilization of body fat and protein lowers FE (Drackley and Reynolds, 2021). In dairy cows NEB begins a few days prior to parturition and extends from 10 to 12 (Butler, 2003) and 17 weeks pp (Garnsworthy, 2007), with the nadir occurring at approx. 14 d pp (Butler, 2003) and this can negatively effects on ovarian activity (Diskin et al. 2003; Friggens and Chagunda, 2005; Chagas et al. 2007; Leroy, et al. 2008; Aguilar-Perez et al. 2009) lowering CR (Butler, 2005) and increasing the PPI to conception (Domecq et al. 1997; Lopez-Gatius et al. 2003; Mäntysaari et al. 2022) increasing infertility (Westwood et al. 2002; Buckley et al. 2003) due to an inadequate nutrient supply that negatively affects follicular growth, conception and pregnancy recognition (Murphy et al. 1991; Diskin et al. 2003; McDougall et al. 2005; Aguilar-Perez et al. 2009).

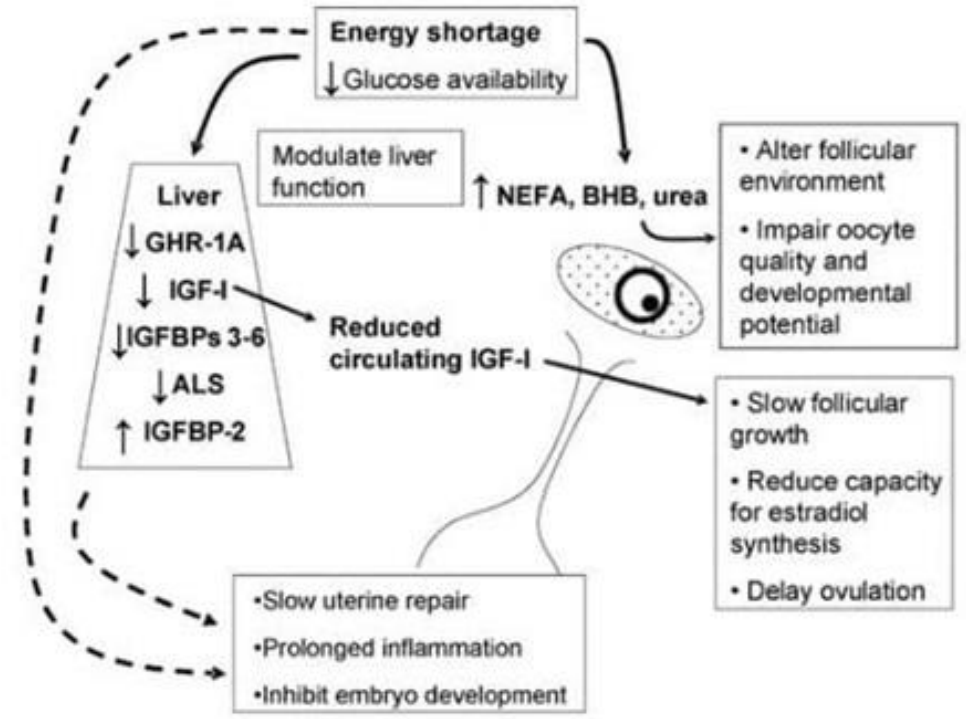


Fig. 1.8: Schematic summary of the effect of energy shortage / negative energy balance (NEB) on fertility through the effect on the liver, ovaries and uterus (Nigussie, 2018)

The physiological mechanisms that link these responses with nutrition include reproductive hormones, growth factors and other metabolites (Webb et al. 2004) that affect an array of chemical messages and metabolic processes involved in the maintenance of EB and reproductive process influenced by the HPG axis (Schneider, 2004). The potential effect of nutrition, in dairy cows and heifers, on ovarian function includes the synthesis and release of GnRH via the hypothalamus; FSH by the anterior pituitary; and LH and GH that regulates follicle growth and steroid synthesis at the ovarian level (Prunier and Quesnel, 2000). In addition, GH facilitates reproduction (Lucy et al. 1999) by indirectly affecting changes in the metabolism of nutrients, affecting insulin sensitivity, IGF-I and IGFBP concentrations (Ramaley and Phares, 1980) that influence the ovary due to the cascading of growth factors and binding proteins (Diskin et al. 2003; Prunier and Quesnel, 2000) (Figure 1.7 and 1. 8). The IGFBPs have been involved in controlling the availability of locally produced IGF-II to ovarian follicles (Webb et al. 2003) and IGFBP-2 and IGFBP-4 concentrations actively modify follicles in direct response to changes in diet (Armstrong et al. 2003). During periods of undernutrition (Fenwick et al. 2008) and/or severe NEB plasma concentrations of IGFBP have been low (Llewellyn et al. 2007) and this limits the availability of IGF to follicular cells, reducing the proliferation follicular cells and steroidogenesis (Webb et al. 1999). Clearly the link

between nutrient supply and reproductive system has been controlled via hormonal regulation via the hypothalamus, anterior pituitary, and ovaries, with the ovaries and hypothalamus releasing hormones that regulate the production of gonadotrophins, which regulate oestrous behaviour and appetite, and sense nutrient availability and somatotrophin inhibit GnRH and subsequent LH production reducing follicular development (Walsh et al. 2011).

The growth and development of ovarian follicles are influenced by NEFA and insulin, IGF-1 and leptin concentrations (Lucy, 2000; Leroy et al. 2010a) among other factors. Negative energy balance (NEB) has been associated with low plasma IGF-1 and LH (Lucy, 2000), insulin, IGF-1 and leptin concentrations (Wathes et al. 2007; Lucy, 2008; Wathes, 2012) and greater plasma NEFA concentrations, directly related to BCS loss, has been associated with poor follicular growth (Vanholderet al. 2006; Walsh et al. 2011; Garcia-Garcia, 2012). Greater MY can place the cow in NEB during early lactation and while GH stimulates IGF-1 production in the liver (Jones and Clemmons, 1995), NEB renders the liver less receptive to IGF-1 due to the downregulation (Donaghy and Baxter, 1996) of the GH receptors (Vicinal et al. 1991) defined as “growth hormone resistance”. An inability to meet the nutrient requirements affect gonadotropin excretion involved in the sustenance of follicular growth and ovulation (Zulu et al. 2002), which include plasma IGF-1 concentrations (Pell, Saunders and Gilmour, 1993; Walsh et al. 2011) that stimulate follicular growth and maintain regular ovarian cyclicity and results in dairy cattle that have plasma IGF-1 concentrations > 50 ng/ml at first insemination being five times more likely to conceive (Taylor et al. 2004), due to EB and the requirements of a gravid uterus as well as the additional demand for glucose, fatty acids and protein for milk synthesis (Leroy et al. 2008; Bauman et al. 2013; Drackley and Reynolds, 2021) in lactating cows.

1.12: Nutrient intake, feed efficiency and fertility

Nutrition has been a key environmental factor affecting the growth rate, puberty and AFC (Margerison and Downey, 2005), MY, FE (Reynolds et al. 2011) and fertility of dairy cows (Canfield et al. 1990; Burke and Roche, 2007; Chagas et al. 2008). Higher milk yields require diets of a greater energy concentration that contain a greater proportion of concentrates, which lowers dietary NDF concentration and methane emissions (Yan et al. 2000; Yates et al. 2000). However, greater MY response to concentrate supplementation has been associated with poorer dairy cow health and fertility (Dillon et al. 2006) and postpartum nutrient intake affects a range of reproductive functions, due to its effect on the production of hormones related to folliculogenesis, fertilization and early embryonic development (Boland et al. 2005; Armstrong et al. 2004; Boland and Lonergan, 2005). These processes are known to be energetically demanding (Garcia-Garcia, 2012) and the brain (Fig 1.8) is instrumental in tempering fertility

to reflect nutrient availability (Scaramuzzi et al. 2006; Evans and Anderson, 2012) and initiate reproductive function when adequate nutrients have been made available (Cameron and Nosbisch, 1991; Casanueva and Dieguez, 1999; Schneider, 2004). Glucose is very important mediator of nutritional effects on reproduction (Omari et al. 2020) and plasma concentrations have been inversely correlated with energy (Yelich et al. 1996) and its transport is facilitated by glucose transporters (**GLUTs**) involved in hypothalamic regulation (Wang et al. 2012) that influence LH secretion through GnRH system (Bucholtz et al. 1996; Roland and Moenter, 2011; Garcia-Garcia, 2012). Alterations in glucose transport and metabolism indicate an interaction between glucose metabolism and GLUTs that affect foetal development in the preimplantation embryo (Leppens-Luisier et al. 2001) and the earliest stages of foetal development (Purcell and Moley, 2009). During such periods of lower feed and/or nutrient intake and NEB reduce plasma concentrations of insulin (Vizcarra et al. 1998) and reduce androgen and oestradiol production (Scaramuzzi et al. 1999) compromising the ability of follicles to acquire LH receptors (Diskin et al. 2003) and respond to LH pulses that increasing the occurrence of atypical cycles.

The glucose demand of dairy cows increases rapidly as corpus milk production is initiated at partition, while dry matter intake (**DMI**) has been lower than maximum during early lactation and unable to meet the glucose requirements and greater hepatic gluconeogenesis and lower glucose oxidation by peripheral tissues allow more glucose to be supplied to the mammary gland to synthesis lactose (Linzell, 1972; Reynolds et al. 2003; Larsen and Kristensen, 2013; Habel et al. 2022) as well as for nicotinamide adenine dinucleotide phosphate reduction during the synthesis of milk fatty acids and milk proteins (Bauman and Griinari, 2003; Liu et al. 2013) and pancreas to supply insulin to the ovary. Recent study shows that glucose balance of dairy cows was close to zero (3.2 ± 13.5 mol C) in the first week of lactation cows were at risk of substantial shortages of glucose for maintenance, potentially affecting fertility (Habel et al. 2022). Feed intake and glucose balance highlight the metabolic association between limited glucose availability during the pp period, when mammary and immune cells simultaneously impose great demands in addition to uterine reformation and other stressors associated with, e.g., calving and animal regrouping (Sundrum, 2015; Habel and Sundrum, 2020; Habel et al. 2022).

The main substrates affecting hepatic gluconeogenesis in ruminants have been propionate from ruminal fermentation, lactate from lactic acid cycling, amino acids (**AA**) from muscle catabolism and glycerol from adipose tissue lipolysis that have been prominent during early lactation (Drackley and Reynolds, 2021; Habel et al. 2022) . Plasma glucose has been derived from hepatic gluconeogenesis of propionate from ruminal fermentation, lactate from lactic acid

cycling and AA from muscle catabolism and glycerol from adipose tissue lipolysis, which has been particularly prominent during early lactation (Drackley and Reynolds, 2021) and the metabolites from the diet and intermediary metabolism serve as precursors. Propionate, which has been produced from long-chain carbohydrates by rumen bacteria, has been a major glucogenic precursor, supplying between 40 and 90% of total glucose carbon for gluconeogenesis (Larsen and Kristensen, 2009; Aschenbach et al. 2020; Habel et al. 2022). These outcomes demonstrate the importance of monitoring and managing BCS of dairy cattle, specifically pre- and pp BCS, to achieve appropriate target BCSP, NEB and change in BCS during early lactation to minimize the effect on fertility and 'carry-over' effects on MY throughout lactation (Garnsworthy et al. 2007; Roche et al. 2009; Wall and McFadden, 2007; Bernier-Dodier et al. 2010; Murney et al. 2015; Torledo et al. 2020).

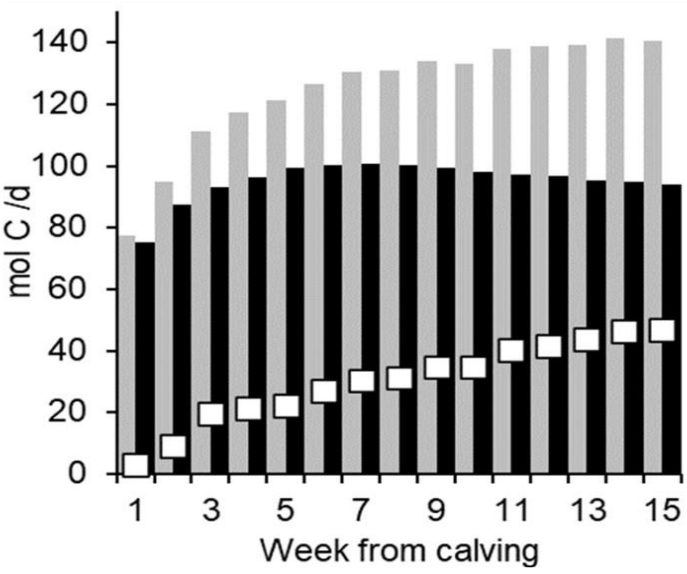


Fig. 1.9: Weekly mean supply (■) and demand (■) for glucogenic carbon and glucose balance (□) of high milk-yielding dairy cows between parturition and 105 d pp (Habel et al. 2022) Note: Supply of digestive and endogenous precursors; Demand by the mammary gland, protein tissue, red blood cells and the brain.

Other precursors from the digestion of feedstuffs encompass glucogenic amino-acids and L-lactate, which are synthesized by rumen microbes (Drackley and Reynolds, 2021), particularly when the diet contains a large proportion of starch (concentrates) (Aschenbach et al. 2020; Habel et al. 2022), lowering acetate:propionate ratio. The availability of these exogenous precursors of gluconeogenesis depends on the rate of transfer from the ruminal and duodenum to the portal vein because propionate has been modified when it has been metabolised within the rumen epithelium (Kristensen and Harmon, 2009; Nozière et al. 2010), while AA can be metabolised as an energy source throughout the digestive tract (Lapierre and Lobley, 2001; Berthiaume et al. 2001; Habel et al. 2022). Glucose absorption has been limited

even in dairy cows offered high (starch) concentrate diets, primarily by the rate of starch hydrolysis in the gut (Huntington et al. 2006; Reynolds et al. 2014; Mills et al. 2017). In addition to the utilization of luminal glucose within the duodenal epithelium, the portal-drained viscera may use 0.3 to 2.2 g/d/kgBW of glucose from arterial supply (El-Kadi et al. 2008; Lapierre et al. 2010). These affect mammalian oocytes and blastocysts that use glucose a major metabolic substrate and deficiencies due to NEB compromise the ability of the oocytes to reach the second metaphase (Domiko and First, 1997) lowering oocyte maturation and embryo viability (Purcell and Moley, 2009; Wang et al. 2012) contributing to the occurrence of atypical ovarian cycles.

The higher yielding dairy herds require higher energy dense diets that include more cereal-based concentrate feeds (Eastridge, 2006), which decrease methane emissions per cow by decreased as proportion of forage in the diet (Yan et al. 2000). However, imported concentrated feeds have a higher carbon footprint compared with home-grown forage (Thomassen et al. 2008), while the quality of forages is extremely important in increasing FE (Drackly and Reynolds, 2021). Energy balance, protein, starch, fat and fatty acid content of the diet affect reproductive competence (Boken et al. 2005; Chagas et al. 2007; Garnsworthy et al. 2008 a, b, c, d; Aguilar-Perez et al. 2009). A meta-regression, showed that increasing ME supply (MJ/d) was associated with shorter PPI to conception, whereas greater differences between ME supply and requirements for milk production was associated with longer PPI to conception (Table 3), with a reduction of 10 MJ/d in negative ME balance predicted to shorten the PPI to conception by 2.87 d (Rodney et al. 2018). Interestingly, milk protein yield which has been greater from a lower acetate : propionate ratio in very early lactation (< 21 d pp) has been positively associated with CR to first service (Rodney et al. 2016), and others identified positive associations between milk protein concentrations and improved reproductive performance (Buckley et al. 2003; Madouasse et al. 2010; Morton et al. 2016), which may be due to greater propionic acid, glucose and AA availability. Short-term changes in diet composition and supplementation affect also affect the follicle size, oocyte maturation, embryo survival and regularity of ovarian cycles following parturition (Bossis et al. 1999; Webb et al. 2004; Adamiak et al. 2005; Robinson et al. 2006; Aguillar-Perez et al. 2009). These affect the efficiency of the reproductive process and fertility both directly and indirectly (Lucy, 2003; Garnsworthy et al. 2009) though increases in plasma insulin and IGF-I concentrations (Gong et al. 2002), which affect gonadotrophin concentrations and stimulation of follicular growth, while suppressing cell apoptosis in ovarian follicles (Jorritsma et al. 2003; Williams et al. 2003; Monget and Martin, 1997). Plasma IGF-I concentrations are not easily modified (Wientjes et al. 2012), but they can be modulated by increasing dietary carbohydrate supply, but are not

affected by fatty acid supplementation (Garnsworthy et al. 2008b). However, the exact insulin profile required to achieve high follicle quality and uniformity is unknown (Wientjes et al. 2012).

The inclusion of greater amounts of dietary starch enhances the ruminal fermentability of starch usually resulting in increased plasma insulin concentrations, which mediates recoupling of the GH/IGF-1 axis (Butler et al. 2003) and has been important for follicle development and ovulation as demonstrated by Gong et al. (2002) when offered cows of low- and high-genetic merit iso-energetic diets that differed in the ability to induce high or low plasma insulin concentrations. However, the evaluation of the effect on nutrition in different experiments can be confounded by changes in MY, composition and changes in BCS and a systematic review by Rodney et al. (2018) identified 39 studies, that used starch, fats/oils, protein and AA, minerals that were evaluated, and four multivariable models were used to estimate the effect of the diet and production variables on CR and PPI to conception, and subsequent CI. Increases in dietary starch or energy density tended to increase PPI to conception, although this occurred in only three experiments and the results in each were variable (Figure 1.10) (Rodney et al. 2018). The use of high-starch diets reduces the length of the PPI to first pp ovulation and resulted in a greater proportion of cows having oestrous cycles within the first 50 d pp, however responses were inconsistent (Garnsworthy et al. 2009) and while higher starch diets increase plasma insulin concentrations, excessive amounts of readily fermentable starch can lower DMI and offset any potential benefits of dietary manipulation on ovarian function. The supplementation of fat in dairy cows diets has been a common strategy to increase the energy density of diets and lower the negative effects of NEB (Palmquist and Jenkins, 2017; Bionaz et al. 2020), which has been effective provided that the DMI and rumen function have been not lowered by fatty acid (FA) isomers that depress milk fat (Chamberlain and DePeters, 2017, 2017; de Souza and Lock, 2019; Manriquez et al. 2019) and the beneficial effect has been related to the type of FA type than greater energy intake (Herrera-Camacho et al. 2011). Improvements in reproductive and immune function associated with PUFA from n-3 (α-linolenic acid, ALA; eicosapentaenoic acid, EPA; docosapentaenoic acid, DPA; and docosahexaenoic acid, DHA) and n-6 (linoleic acid, LA; and arachidonic acid) have receives more research (Moallem, 2018; Moallem et al. 2020) and more recent work showed that pp rumen-protected linseed oil intake and plasma n-3 PUFA concentrations modulated adiponectin expression and improved reproductive parameters (Pereira et al. 2022).

In terms of dietary strategies such as supplemental fats/oils have more consistently decreased methane per kg feed DMI and often increase MY (Beauchemin and Grainger, 2011), but in this case, the effects on MY have been through mechanisms independent from the effects on

methane emissions. Other ways that improving feed efficiency may decrease methane output per unit of milk produced relate to increasing rates of nutrient passage through the rumen, shifting site of digestion from the rumen to the intestine, feeding supplemental fat/oil, or reducing heat production by the animal by alterations in metabolism that result in greater MY (Drackley and Reynolds, 2021). Methane production has been related more to the populations of hydrogen producing bacteria (Wallace et al. 2017). During the transition period the inclusion of fat/oil supplements have been found to improve pp reproductive performance (Thatcher et al. 2006; De Veth et al. 2009), by reducing NEB and incidence of metabolic disease (von Soosten et al. 2012) by increasing the energy density (**M/D**) of the diet without the inclusion of greater amounts of rapidly fermentable energy (**FME**) from cereals. Lower dietary crude protein concentrations and intake by dairy cows can reduce GHG emissions from imported feeds, which can have a limited effect on MY (Reynolds et al. 2016), while greater AA intake, particularly lysine and threonine and milk protein yield tend to be associated with increased PPI to conception (Rodney et al. 2018). While greater phosphorus concentration magnesium and non-fibre carbohydrates (**NFC**) such as starch, sugar and pectin from forages and cereals tend to be associated with decreased PPI to conception (Rodney et al. 2018). This was not surprising because energy intake accounts for 86% of the variation in dietary energy intake of dairy cows (Moe et al. 1972). Increases the propionate to acetate ratio can be achieved by improving forage quality and increasing starch and protein concentrations, while lowering the fibre content by optimising maturity and nutrient content at harvest and fibrolytic activity in the rumen (Drackley and Reynolds, 2021).

Herd replacement rate has one of the greatest economic and environmental impacts on dairy systems (£14 per cow) and ability to reduce CO_2-eq. per cow (ranging from −48 to −53 kg) and per unit milk solids (ranging from −91 to −98 g/kg) per one percent increase (Bell and Wilson, 2018). The measurement of enteric CH_4 emission from growing dairy heifers has been limited and yielded variable results (Cottle et al. 2011). Early work showed that methane emissions from dairy herd replacements were 10 g/h per 500 kg live weight (Crutzen et al. 1986; Lockyer, 1997), while later work showed that dairy heifers had annual CH_4 emissions of 31 kg per animal from birth and 12 mo. and 75.5 kg between 12 and 24 mo. of age, averaging 57 kg / animal (Dall-Orsoletta et al. 2019). While Jiao et al. (2014) found that grazing Holstein Friesian heifers CH_4 emissions per animal were 36.2 kg from birth to 12 mo. and 64.3 kg between 12 and 24 mo. of age. While a unit reduction in enteric CH_4 would notably reduce CO_2-eq. emissions per cow (ranging from −48 to −50 kg) and per unit milk solids (ranging from −75 to −79 g/kg), if selection for lower methane emitting animals was possible commercially (Bell and Wilson, 2018). While improving the digestibility of cow rations can lower enteric CH_4 emission (Garnsworthy, 2004; Wilkinson and Garnsworthy, 2017), which can be achieved

through plant management, feed processing, diet formulation and the addition of feed additives and supplements (Knapp et al. 2011 and 2014). While higher feeding levels tend to make dairy heifers hyper insulinemic and impair oocyte quality when they have been in moderately high BCSP, however, increasing feeding level improves the blastocyst yield of heifers in lower than target BCSP (Adamiak et al. 2005), which should be approx. 0.5 greater than BCSP than that recommended for cows (Margerison et al. 2022). Factors that affect FE include feed intake, digestion, metabolism associated with BCS associated fat and protein deposition, physical activity, thermoregulation, and other components of basal metabolism (Drackley and Reynolds, 2021) and these factors are important in the evaluation of factors related to the fertility of dairy cows.

1.13: Conclusions

Enteric fermentation by ruminants contributes 44.3% of global livestock emissions (FAO, 2018) and as the world population is forecast to increase, the concomitant increase in food production need to be supplied by applying agricultural practices that minimize the effect they have on climate change and so this thesis aims to estimate the potential effect on the Co2 eq. from the production of milk. The fertility of dairy cows is a main factor that may affect the CO_2-eq. of milk production systems, due to infertility related extended CI and low PR, which lower lifetime MY and increase dairy herd RR. Dairy farming systems need to reduce the CO_2 eq. and greenhouse gas emissions per unit of dairy product (milk, fat and protein) produced, which has successfully been achieved by increasing the MY of dairy cows, by diluting the maintenance requirements of the animal, therefore increases feed efficiency, but this reduces the regularly of ovarian cycles and extends the PPI to conception and CI, while lowering PR and RR, which may be related to greater NEB.

1.14: Aims and objectives of this thesis

This thesis aimed to assess the occurrence rate and factors that were associated with the occurrence of atypical oestrus cycles (ACL) in high yielding Holstein Friesian dairy cows and assess the impact infertility has on CO_2-eq. emissions from dairy production. The objectives of this thesis are to use milk P4 concentrations to evaluate the occurrence of ACL and assess the effect of ACL on the fertility and CI and RR related CO_2-eq. emissions of high yielding Holstein Friesian dairy cows and compare the fertility, milk yield, feed intake (DMI) and FE of cows, including the BCS of cows at parturition and loss of BCS following parturition that had ACL and typical ovarian cycle length (TCL) and evaluate how CO2-eq. emissions from dairy production can be reduced.

Chapter 2.0

Experiment 1

Occurrence of atypical ovarian cycles and the effect on the calving interval, pregnancy, and replacement rates, and infertility-related carbon emissions from high-yielding dairy cows

Occurrence of atypical ovarian cycles and the effect on the calving interval, pregnancy, and replacement rates, and infertility-related carbon emissions from high-yielding dairy cows

2.1: Abstract

Reproductive performance of dairy cows continues to be a major concern of the dairy industry worldwide and the postpartum interval (PPI) to conception and pregnancy rate (PR) and calving interval (CI) are important parameters affecting the economic and environmental impact of the dairy industry. This study aimed to assess the occurrence and effect of atypical reproductive cycle length (ACL) on the PPI to conception and PR and the effect on GHG emissions from dairy cows using 230 high-yielding Holstein Friesian dairy cows, which were located according to pp cycle length to cows that had ACL and typical cycle length (TCL). The majority, 69 %, of cows had TCL, however, a high proportion, 31%, of cows exhibited ACLs, which included 0.20% that had prolonged pp luteal cycle length ≥ 20 d, followed by 0.07% of cows that exhibited the delayed onset of ovarian luteal activity and 0.04% the cessation of luteal activity. Cows with ACL were of a greater parity (P=0.032) and had a greater PPI to first progesterone (**P4**) rise (P<0.001), lower CR to the first insemination (P<0.034), PPI to conception (P<0.001), and longer PPI to conception (P<0.001) and CI (P<0.001), while PR and BCS at parturition did not differ. The occurrence of ACLs increased the carbon footprint (CO_2-eq) produced by additional RR (P<0.001) and CI above 365 d and 375 d increased the CO_2-eq produced (P<0.001), and the CO_2-eq production related to infertility (P<0.001), which is the combined CO_2-eq for additional herd replacements and CI. In conclusion, 31% of high-yielding dairy cows exhibited ACLs, which increased the PPI to the first insemination, lowered the conception to the first insemination, and increased the PPI to conception and CI of dairy cows. Infertility substantially increased the CO_2-eq of dairy cows due to cows that had ACLs having a longer PPI to first P4 rise, first insemination and conception, which increased the CI.

Keywords: Dairy, Cycles, Calving interval, Replacements, Carbon.

2.2: Introduction

Reproductive performance is a major concern of the modern dairy industry worldwide, due to its detrimental effect on farm profitability (Gröhn and Rajala-Schultz, 2000; Horn *et al*. 2012; De Vries, 2017; Kim et al. 2019). It is believed that annual calving intervals are an optimal indicator of fertility and profitability of dairy herds (Pryce et al. 1997; Inchaisri et al. 2010; Bell and Wilson, 2018), which requires a postpartum interval (**PPI**) to conception to successfully occur within 85 d pp (Grimard et al. 2006). The biological possibility of conception in this time frame requires a coordinated function of the hypothalamus, pituitary, ovaries, and uterus,

resulting in uterine involution and timely resumption of ovarian function (Deori and Arundhati, 2015; Gautam et al. 2009; Negasee, 2020). In addition, diverse factors, e.g., body condition score (**BCS**) at parturition and changes in BCS during the transition, pp and/or service period, parity, milk yield, calving to first service interval, and peripartum disorders such as dystocia, metritis, and retained placenta (Youngquist and Shore, 1997; Smith et al. 2002; Walsh et al. 2011) have been shown to extend the PPI to conception (Grimard et al. 2006; Siddiqui et al. 2013).

The PPI to conception, sometimes referred to as the days open are important parameters that are used to evaluate the reproductive and economic performance of the dairy herd (Harman et al. 1996), and increases in the PPI to conception are of an economic disbenefit to the dairy industry, lowering the milk yield in relation to labour and feed costs (Inchaisri et al. 2010), income from the number of calves born (Cabrera, 2014) and lifetime productivity of cows (González-Recio et al. 2004), while increasing breeding costs (González-Recio et al. 2004; Cabrera, 2014) culling rates (González-Recio et al. 2004; Inchaisri et al. 2010; Cabrera, 2014) and RR. Greater genetic merit for milk yield (**MY**) has been cited to be responsible for 13 to 30% of the observed variance in pp ovarian activity (Veerkamp et al. 2000; Royal et al. 2002b; Petersson et al. 2007) and delayed first ovulation (Petersson et al. 2006; Cutullic et al. 2011; Piccand et al. 2013). Greater MY (Pryce et al. 1997; Brito et al. 2021) has been shown to be associated with greater pp BCS loss and negative energy balance (**NEB**), which negatively affects the PPI to the onset of ovarian activity, oocyte quality, and CR due to endocrine regulation of changing lipogenesis to lipolysis during early lactation (Garcia-Carcia, 2012).

Increasing MY per cow has been successful in achieving greater feed efficiency (**FE**) and the dilution of enteric **GHG** emissions (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014), which is essential in maintaining global warming < 1.5°C up to 2030 and achieving net zero emissions by 2050 (UN, 2022). However, the deleterious effect of high genetic merit for MY on the regularity of ovarian cyclicity (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and pregnancy rates (**PR**) (Brito et al. 2021) lower the length of the productive life of dairy cows (Knaus, 2009), which has been shown to increases GHG emissions related to greater calving interval (**CI**) (Bell and Wilson, 2018) and lower PR (Brito et al. 2021) that increases the amount of herd culling due to infertility (Pryce et al. 1997) and replacement rate (**RR**) (Zehetmeier *et al.* 2014; Bell *et al.* 2015) increasing the number of herd replacements required. This would have a negative effect on the cost of milk production (Yusuf et al. 2011; Horn *et al*. 2012; De Vries, 2017; Bell et al. 2015) and the carbon footprint of dairy production systems due to a greater number of herd replacements on farm (Garnsworthy et al. 2004; Bell and Wilson,

2018), however, there are few published studies with the exception of modelling (Crutzen et al. 1986; Garnsworthy et al. 2004; Bell and Wilson, 2018) that have collected data to assess the factors related to the occurrence of atypical ovarian cycles length (**ACL**) and its effect on the PPI to conception, pregnancy and replacement rates of higher-yielding dairy cows that has the potential to increase FE and lower CO2-eq of dairy production systems by diluting the feed and maintenance costs of milk production in the UK.

2.3: Aim and objectives

The aim of this study was to use milk P4 concentrations to assess the ACL occurrence rate in high-yielding (>11,000 L/cows/year) dairy cows and assess the effect on CI and RR. The objectives were to assess PPI to the first pp rise in P4, commencement of ovarian activity, evaluate ACL occurrence rates and the effect of ACLs on the PPI to conception and CI, CR and PR, and use this data to estimate the effect of the CI and RR on CO2-eq.

2.4: Materials and methods

2.4.1: Study location and ethical approval

This study was carried out using P4 analysis and data that had been previously collected at the University of Nottingham Centre of Dairy Science and Innovation (CDSI), Sutton Bonington, Leicestershire, UK, by George Mann and Morag Hunter, between January 2009 and June 2013 in accordance with the approval and procedures of the University of Nottingham Animal Ethics Committee and authority of the UK Animal (Scientific Procedures) Act 1986, which was obtained prior to the commencement of the study.

2.4.2: Experimental design and animal management

This study was carried out using 228 Holstein-Friesian dairy cattle that were selected at random from the 230 cows that calved annually at the Centre of Dairy Innovation (CDSI) of the University of Nottingham, which had an average annual MY of 11,500 L/cow. In this study the cows were randomly selected from an all year-round calving herd according to calving date to provide a representative sample ≈33.3 % of all cows that calved in the herd over a three-year period. The cows used in this study had a mean parity of 2.0 (± 0.41) (from 1 to 8), live weight of 710 (± 24.1) kg and BCS of 2.65 (± 0.46) (from 2.25 to 3.50) at parturition and the cows were allocated retrospectively according to pp luteal activity into one of two groups that had; typically normal cycle (≤ 20 d) (**TCL**) and atypical prolonged luteal cycle length (≥ 21 d) (ACL) according to Lamming and Darwash (1998). This was undertaken annually over a two day period using P4 concentrations of milk samples that were collected thrice weekly (Petersson et al. 2006; Cutullic et al. 2011) according to two consecutive milk samples P4

concentrations that were greater than 3 ng/ml indicated the presence of luteal activity (Table 2.1).

Table 2.1: Application of milk progesterone (P4) concentrations used to categorise atypical ovarian activity of lactating dairy cows

Atypical reproductive cycle pattern	Definition
Delayed ovulation	
Type I	<3 ng/ml ≥45 d pp
Type II (prolonged inter-luteal interval)	<3 ng/ml ≥12 d between two luteal phases
Delayed luteolysis (persistent corpus luteum)	
Type I: During first cycle	>3 ng/ml ≥19 d during first pp oestrous cycle
Type II: Subsequent cycles prior to insemination	>3 ng/ml ≥19 d during subsequent pp cycles
Late embryonic mortality >16 d post-insemination	>3 ng/ml ≥19 d following insemination, followed by lower P4 levels

Ammended from Lamming and Darwash, 1998

2.4.3: Animal housing, feeding and management

The cows were housed in the same pen, using multiple (n=9) groups of 50 cows per pen, which had 55 free stalls in each pen that were fitted with cantilever divisions and rubber matrasses, which were cleaned and dusted with fresh sawdust daily. The feeding alleys were fitted with rubber covered concrete, which were cleaned at 4 h intervals by automatic above ground scrapers. All animals had ad libitum access to clean fresh water and a partial mixed ration PMR that consisted of grass, maize and whole crop silage (50: 25: 25) on a DM basis and protein meal, which was formulated to supply adequate nutrients for animal maintenance requirements, plus 32 L of milk production per day (Table 2.2 a, b). During milking, cows were offered a pelleted dairy compound feed through the milking stations integral feed bin (Lely Astronaut A3; Lely UK Ltd., St Neots, UK), which was distributed at 2 kg/hd/d from parturition, which increased at 0.2 kg/d up to a maximum of 12 kg /hd/d up to 60 d pp, followed by which allocation remained the same until 100 d pp, followed by which the feeding rate was reduced according to individual animal MY, which was offered at 0.4 kg/L. This resulted in an average forage to concentrate ratio of 1.3:1.

2.5: Measurements

2.5.1: Milk progesterone concentration

Milk samples (20 ml) were collected automatically via a Lely Shuttle connected to the robotic milking station for progesterone analysis, in the morning, thrice weekly from individual cows between 7 and 130 (± 0.3) d pp. Milk samples had a broad spectrum Microtabs®II (containing Bronopol and Natamycin; D&F Control Systems, Inc., USA) added and were frozen -22 ° C until being thawed for 24 h at room temperature prior to the determination of progesterone concentration using an enzyme-linked immunosorbent assay (**ELISA**) (Ridgeway Science Ltd., Alvingdon, UK) in accordance with Bedere et al. (2016). The coefficients of variation between assays for ELISA on 5 ng/mL control samples ranged between 8 and 10%. Luteal activity was determined using two consecutive milk P4 concentration thresholds according to Petersson et al. (2006) as adapted by Cutullic et al. (2011) to establish baseline progesterone concentrations and distinguish between P4 luteal phase concentration thresholds low < 5 ng, high > 5 ng and intermediate ≈ 5 ng, while increases in P4 concentrations between consecutive milk samples were considered to be due to corpus luteum (**CL**) activity and decreased P4 concentrations indicated luteolysis of the CL. These were used to distinguish luteal and inter-luteal phases and calculate the PPI to first P4 rise, ovulation, inter-ovulatory luteal cycle length and PPI to conception according to Cutullic et al. (2011). The first day of high P4 concentrations >3 ng/ml that satisfied the requirements was determined to be the PP interval prior to the onset of luteal activity according to the technique presented in (Table 2.1) and the PPI to the first PP ovulation was determined by subtracting an expected 5.7 days from the recorded delay to the PPI to first P4 concentration increase in accordance with Darwash, Lamming and Wooliams (1997).

2.5.2: Diet composition, feed sample collection and feed analysis

Samples (500 g) of all diet components; concentrated feed, forages and pelleted dairy compound feeds were collected weekly and stored at -20 °C until being thawed at room temperature for 12 h until analysis. Individual grass, maize and whole crop cereal silages were analysed by near-infrared spectroscopy using forage analysis assurance group equations to predict DM content, nutrient content, and silage characteristics (Sciantec Ltd., Yorkshire, UK). All feed samples were analysed using AOAC (1990) methods for dry matter, CP, NDF, starch, sugars, and fat (oil acid hydrolysis; OAH), which were determined directly, whilst metabolizable energy was calculated as (0.14 × NCGD + 0.25 × OAH), in which NCGD = neutral cellulase plus gamanase digestibility that was assessed according to the feeding stuffs (England) regulations (FRS) 2005, using statutory instrument No. 3281 (Sciantec Ltd., Yorkshire, UK) (Table 2.2).

Table 2.2: Partial mixed ration ingredients and composition (± SE) of mixed ration and pelleted concentrate offered at milking

Ingredients (kg DM/d/cow)	Partial mixed ration	Pelleted concentrate
Diet components		
Maize silage, g/kg DM	275 (± 55)	-
Grass silage, g/kg DM	229 (± 48)	-
Whole crop silage, g/kg DM	209 (± 42)	-
Wheat straw, g/kg DM	9 (± 0.10)	-
Soya: Rape seed meal, g/kg DM	174 (± 23)	-
Sugar beet pulp, g/kg DM	22 (± 2.2)	
Molasses, g/kg DM	27 (± 2.8)	-
Protected fat, g/kg DM	19 (± 1.6)	-
Dried distillers grains with solubles	7 (± 0.6)	-
Soya hulls, g/kg DM	7 (± 0.9)	-
Minerals and vitamins, g/kg DM[1]	22 (± 0.01)	-
Total	1000 (± 0.00)	-
Diet composition		
Dry matter, %	45.2 (± 0.10)	86.5 (± 0.01)
CP, % DM	16.8 (± 0.09)	18.0 (± 0.01)
ME, MJ/Kg DM	11.8 (± 0.02)	12.5 (± 0.01)
NDF, % DM	35.5 (± 0.01)	21.0 (± 0.01)
Starch and sugar, % DM	26.2 (± 0.05)	52.0 (± 0.01)
Oil, % DM	4.5 (± 0.04)	5.0 (± 0.01)
Ash, % DM	7.5 (± 0.03)	
CO_2-eq. / kg, kg	0.33 (± 0.012)	1.32 (± 0.010)

[1]-Dietary minerals and vitamins; calcium, 18%; phosphorus, 10%; magnesium, 5%; salt, 17%; copper, 2,000 mg/kg; manganese, 5,000 mg/kg; cobalt, 100 mg/kg; zinc, 6,000 mg/kg; iodine, 500 mg/kg; selenium, 25 mg/kg; vitamin A, 400,000 IU/kg; vitamin D3, 80,000 IU/kg; and vitamin E, 1,000 mg/kg.

2.5.3: Oestrus detection, artificial insemination and pregnancy diagnosis

Oestrus was detected using activity monitors to detect oestrus-related behaviour using acceleration technology that was attached to the neck collar of individual cows, which continuously measured horizontal acceleration related to upward movements of head and neck during walking and mounting behaviour to calculate a daily activity index. This index was interpreted using specially developed algorithms based on deviations from typical stored

activity patterns, to separate increased activity associated with oestrous behaviour. This activity was automatically transferred via an antenna fitted to the robotic milking station to herd management software that provided graphic indication and lists of individual cows to identify the reproductive, rumination and health status (Lely Astronaut A3; Lely UK Ltd., St Neots, UK). Artificial insemination was undertaken 12 h post onset of increased oestrus activity, prior to ovulation, by a professional breeding technician (Genus Breeding Ltd, Nantwich, UK) according to animal activity. The existence of pregnancy was confirmed by ultrasound (**US**) observations that were carried out at 18 to 21 following a successful AI using a Voluson *I* ultrasound device (General Electric Healthcare Systems, Vienna, Austria), which was fitted with a 12L RNA linear array, multifrequency transducer that was operated between 4 and 12 MHz. The ultrasound machine depth, echo-amplification, persistence, output power etc. settings were retained and used for all US examinations. This pregnancy was confirmed by rectal palpation no earlier than 40 to 60 d of gestation by a qualified veterinarian.

2.5.4: Carbon dioxide equivalents of calving interval and replacement rate

The CI was calculated using the PPI to conception with the addition of a standard gestation period for lactating dairy cows of 283 d. The RR related to infertility was calculated using the PR (100 – PR = RR). The CO_2-eq. emission per day of CI, was calculated using 14.9 kg/d, which was representative of the average amount CO_2-eq. calculated per d of CI for dairy cows in the UK by Bell and Wilson (2018) plus its effect on a RR using an assumed RR of 20% and CI of 365 d. The CO_2-eq emissions related to RR was calculated using the % RR x 48.7 kg, which was the CO_2-eq emissions calculated per % RR for dairy heifers managed under UK conditions by Bell and Wilson (2018). The CO_2-eq for CI and RR were added together to calculate the CO_2-eq. that was related to infertility. In this study, the CO_2-eq. per % of RR and CI were calculated for a 365 and 375 d CI.

2.5.6: Statistical analysis

The data was collated in Microsoft Office Excel 2016 and used to calculate PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR, PPI to conception and this was used to calculate the CI using a gestation period of 283 d. The data was assessed using the Anderson-Darling test for normality of distribution using Minitab statistical software (Version 17.2.1, 2016, State College, PA) and found to be normally distributed. The occurrence of ACL, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, PPI to conception, CI, CR, PR and CO_2 eq. were analysed using the general linear model (GLM) ANOVA by each variable/ response, initially including year, ACL and TCL as fixed effects and

animal as a random effect in the model. Year was found to have no effect on occurrence of ACL and was removed from the model. The number of inseminations and conception were assessed using Chi square test using ACL and TCL as a row and response as a column the table. Data was reported as means (± SE) and P values, and the existence of significant differences between means were reported at a $P<0.05$ and tendencies were reported at a $P<0.010$.

2.6: Results

2.6.1: Reproductive performance

The majority, 69 %, of cows had TCLs, however a high proportion, 31%, of cows exhibited ACLs, which included 20% that had prolonged pp luteal cycle length ≥ 20 d, followed by 7% of cows that exhibited the delayed onset of ovarian luteal activity and 4% the cessation of luteal activity (Table 2.3). The cows that had ACL were of a greater parity and had greater PPI to the first P4 rise, PPI to conception, lower CR to first service and longer CI. The BCS at parturition and PR did not differ between cows that had ACL and TCL.

Table 2.3: Mean (±SE) parity, body condition (range) and postpartum interval to conception, conception and pregnancy rate and calving interval of high yielding dairy cows that had atypical and typical luteal cycles

	Cycle length		
	Atypical	Typical	P value
Observations, No. cows	71	157	-
Parity, No.	3.2 (±0.25)	2.7 (±0.12)	0.032
Body condition score (0 to 5), 7 d pp	2.70 (0.071)	2.60 (0.040)	0.356
Range in body condition (0 to 5), 7 d pp	2.25 to 3.50	2.25 to 3.00	0.457
Interval to first P4 rise, d pp	37.7 (±2.61)	27.6 (±0.80)	<0.001
Postpartum interval to first insemination, d	77.7 (±3.11)	66.2 (±1.71)	<0.001
Conception to first insemination, %	24 (±0.2)	38 (±0.4)	0.034
Postpartum interval to conception, d	129 (±14.0)	92 (±5.4)	<0.001
Pregnancy rate, %	78 (±0.9)	89 (±0.9)	0.121
Calving interval, d [1]	412 (±40.6)	375 (±22.3)	<0.001

[1] – Calculated from the PPI to conception, plus a gestation of 283 d for all pregnant animals

2.6.2: Effect of fertility on carbon dioxide equivalents

The occurrence of ACLs increased CO_2-eq. produced by additional herd replacements by 535 kg and CI above 365 d increased the CO_2-eq. produced by 566 kg and 375 d by 551 kg. The CO_2-eq. production related to infertility, which is the combined CO_2-eq. for additional herd replacements and CI was 1,101 kg greater for a 365 d CI and 1086 kg greater for a 375 d CI form cows exhibited ALC compared with TCLs (Table 2.4).

Table 2.4: Carbon equivalents related to herd replacements, calving interval (±SE) and infertility of high yielding dairy cows that had atypical and typical luteal cycles

	Cycle length		
	Atypical	Typical	P value
Replacement rate, %	22 (0.2)	11 (0.1)	0.121
CO_2-eq. for herd replacements, kg	1071 (12.9)	536 (6.4)	<0.001
No. days over CI 375, d	37 (2.51)	0 (1.1)	<0.001
CO_2-eq. for days over a CI of 375 d, kg [1]	551 (4.9)	0 (1.2)	<0.001
CO_2-eq. infertility for a CI of 375 d, kg [2]	1622 (13.2)	536 (5.8)	<0.001
No. days over CI 365, d	47 (4.6)	10 (1.2)	<0.001
CO_2-eq. for days over a CI of 365 d, kg [1]	700 (12.4)	134 (9.8)	<0.001
CO_2-eq. infertility for a CI of 365 d, kg [2]	1771 (15.2)	670 (6.3)	<0.001

[1] – Calculated using an assumed replacement rate 20% and CI of 365 d used to calculate additional replacements. [2] – Combined CO2eq for additional herd replacements and calving interval greater than 365 and 375 days.

2.7: Discussion

2.7.1: Reproductive performance

The detrimental effect of increased MY on reproductive performance of dairy cows is the major concern (Gröhn and Rajala-Schultz, 2000; Horn et al. 2012; De Vries, 2017; Kim et al. 2019). Unfortunately, a relatively large proportion of high yielding cows in this study exhibited ACLs (Table 2.3), and a small proportion of cows even had a total cessation of luteal activity. The proportion of cows exhibiting ACL was lower than the approx. 40% of the dairy cow population that have been reported to be affected by ACL that typically delayed commencement of luteal activity (Petersson et al. 2006; Windig et al. 2008; Cutullic et al. 2011), which was apparent in

this study. Researchers have shown that the variance in cycle length and delayed return to ovarian activity was related to the genetics in 13 to 30% of individual cows and that it was negatively genetically correlated with increasing MY (Veerkamp et al. 2000; Royal et al. 2002b; Petersson et al. 2007). The MY of dairy cows continues to increase in developed and developing countries (Margerison 2022) and is related to increased feed efficiency and lower CO_2-eq of milk production (Garnsworthy et al. 2020) due to a dilution of nutrient requirements for maintenance (Capper et al. 2013) making the occurrence of ACL, effect, and relationship with MY and FE need to be evaluated in further research. It is especially important to study this in Holstein Friesian, not only because this breed show more abnormal cyclicity compared with some other breeds, such as Jersey, especially in terms of delayed PPI to first ovulation (Petersson et al. 2006; Cutullic et al. 2011; Piccand et al. 2013), but more importantly because this breed is most commonly used for milk production globally and for cross breeding to increase MY in developed and developing countries (Margerison, 2022).

The deleterious effect of high genetic merit for MY on ovarian cyclicity is, however, complex and has been related to other factors which include the body condition score, health and nutrition of dairy cows (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008). The body condition score (BCS) during transition and at parturition (Young and Shore, 1997; Smith et al. 2002; Walsh et al. 2011) that can extend the PPI to conception (Grimard et al. 2006; Siddiqui et al. 2013) however this is unlikely to be a factor that has affected ovarian activity and conception in this study because the cows that exhibited TCL and ACL in this study received similar transition nutrition and the BCS did not differ at parturition and was well withing industry recommendations (AHBB, 2020). However, excessive pp BCS loss and NEB can equally affect the onset of ovarian activity (Pryce et al. 2001; Garnsworthy, 2006; Patton et al. 2007), affect oocyte quality and CR due to endocrine regulation of changing lipogenesis to lipolysis during early lactation (Garcia-Carcia, 2012) and while this was not assessed in this study it would be important to evaluate BCS at parturition and pp change in BCS in subsequent studies.

2.7.2: Effect of fertility on carbon dioxide equivalents

The maintenance of regular annual calving is an optimal indicator of fertility, profitability (Pryce et al. 1997; Inchaisri et al. 2010) and lower CO_2-eq. emissions of dairy herds (Bell and Wilson, 2018). The PPI to conception or days open are important parameters that are used to evaluate the reproductive and economic performance of the dairy herd (Harman et al. 1996) and minimizing the days open is economically beneficial in increasing milk yield in relation to labour and feed costs (Inchaisri et al. 2010), increasing the number of calves born (Cabrera, 2014) and lifetime productive days (González-Recio et al. 2004). This PPI to conception successfully

occur within 85 d pp (Grimard et al. 2006), however the cows that had ACL in this study had a greater PPI to conception, lower CR to first service and longer CIs. This greater PPI to conception and CI increased the CO_2-eq. associated with additional days of the CI, which is associated with greater breeding costs (González-Recio et al. 2004; Cabrera, 2014) and culling rates (González-Recio et al. 2004; Inchaisri et al. 2010; Cabrera, 2014). The cows that had ACL had lower CR and PR increased the number and the amount of CO2eq associated with raising herd replacements (Bell et al. 2011; Bell, 2015; Bell and Wilson, 2018), which increased the CO_2-eq. substantially and this requires closer evaluation in subsequent studies to see if this is consistently the case between studies.

Overall infertility in this study had a substantial impact on CO2-eq. and GHG emissions from the dairy industry, which warrants further investigation, specifically looking at high MY dairy cows. This is because increasing the genetic merit of dairy cows to increase MY has a deleterious effect on ovarian cyclicity (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and PR (Brito et al. 2021), which subsequently lowers the length of cow productive life (Knaus, 2009) and increases GHG emissions related to greater CI (Bell and Wilson, 2018). It is essential to evaluate the amount of herd culling due to infertility (Pryce et al. 1997) along with CI. These substantially increase the number of replacements required (Zehetmeier *et al.* 2014; Bell *et al.* 2015), which had the most substantial impact of CO_2-eq related to milk production and this needs to be evaluated in more detail, using P4 evaluation of ovarian cyclicity including component corrected MY and FE, which need to include pp change in BCS in high yielding Holstein Friesian dairy cows.

2.8: Conclusions

A relatedly high proportion of high yielding dairy cows exhibit ACLs, which increased the PPI to first P4 rise and conception and lowered conception and pregnancy rates. This increased the calculated CI and RR, which increased the CO_2-eq., partially due a longer CI and a considerably greater need for herd replacements.

2.9: Further work

The substantial occurrence and impact of ACL on CO_2-eq., mainly due to a greater RR, and lesser extent CI requires further study. This needs to include a wider range of factors that may affect the cycle length and fertility of dairy cows, which includes MY and BCS at parturition and pp change in BCS. It is essential individual animal DMI is measured so that FE and CO_2-eq. in relation to component corrected MY can be measured and potentially lowered in the future.

Chapter 3.0

Experiment 2

Effect of atypical ovarian cyclicity on feed efficiency, replacement rates, and carbon equivalent of milk produced by high-yielding dairy cows

Effect of atypical ovarian cyclicity on the fertility, milk yield, feed efficiency, and carbon dioxide equivalent of milk production from high-yielding dairy cows

3.1: Abstract

Increasing the milk yield (**MY**) of dairy cows has successfully increased feed efficiency (**FE**) and lowered the carbon footprint (**CO_2 eq**.) of milk production. However, greater MY has been associated with lower pregnancy rates (**PR**), which is a major concern for the global dairy industry due to its potential to increase the financial cost and CO_2-eq. of milk production related to the need for greater numbers of replacements. This study aimed to evaluate the combined effects of MY, FE, and fertility-related to atypical ovarian cyclicity (**ACL**), such as greater interval to ovarian activity, calving interval (**CI**) and replacement rate (**RR**) due to lower PR and the effect on CO_2 eq. of milk production by assessing the effect of ACL and typical cycle length (**TCL**) on the CI, PR, and infertility-rated herd RR and its effect on MY, FE and CO_2-eq. of milk production. Cows that had ACL (31%) had a greater PPI to first progesterone rise (P=0.003) and to conception and CI (P=0.005) at 160 d pp, but not 130 d pp which showed poor fertility in cows with ACL and TCL at 130 d pp. The CR and PR did not differ at 130 d and 160 d pp between cows with ACL and TCL. Dairy cows that exhibited ACLs had greater fat and protein corrected milk (**FPCM**) (P<0.001) and milk protein (**MP**) yield (P<0.001) and were more FE (FPCM P=0.015; MP=0.002) but had greater CO_2 eq. emissions mainly due to a greater CI (P= 0.010). Body condition score (BCS) at parturition and pp change in BCS did not differ between cows that had ACLs and TCL and were well within industry recommendations. It was concluded that cows that exhibit ACLs had greater FPCM, MP yields, and FE (FPCM and MP yield), had longer PPI to conception and CI at 160 d pp, which was not related to BCSP and pp change in BCS. Infertility-related CI increased CO_2 eq. emissions.

Keywords: Dairy, fertility, feed efficiency, replacements, emissions

3.2: Introduction

It is essential to lower global warming by maintaining the increase in temperature to < 1.5°C up to 2030 and to achieve net zero emissions by 2050 (UN, 2022). In these terms, increasing the individual milk yield (**MY**) of dairy cows has been successful in lowering the enteric greenhouse gas **(GHG)** emissions, by increasing feed efficiency (**FE**) (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014). Unfortunately, increasing the genetic merit of dairy cows for MY has a deleterious effect on the regularity of ovarian cyclicity (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and pregnancy rates (**PR**) (Brito et al. 2021).

Greater MY extends the PPI to conception, which increases GHG emissions related to greater calving interval (**CI**) (Bell and Wilson, 2018), while lower PR (Brito et al. 2021) shortens the length of the productive life of dairy cows (Knaus, 2009) and increases the amount of herd culling due to infertility (Pryce et al. 1997). This increases the herd replacement rate (**RR**) and the number of replacements required (Garnsworthy, 2004; Zehetmeier et al. 2014; Bell et al. 2015), which increases the land, fertilizer, feed, and CO_2-eq. related to the maintenance of non-lactating animals.

The modelling of agricultural systems has shown that the maintenance of greater numbers of herd replacements has been shown to have a substantial negative effect on the carbon footprint of dairy production systems (Garnsworthy, 2004; Bell and Wilson, 2018) along with the results of Experiment 1. Greater herd culling and replacement rates substantially affect the financial cost of milk production (Yusuf et al. 2011; Horn *et al*. 2012; De Vries, 2017; Bell et al. 2015) for farmers. However, there are few published studies that have collected biological data to assess the occurrence of atypical ovarian cycle length (ACL) and the effect on the fertility of high-yielding dairy cows, which include the individual animal evaluation of a wider range of important biological factors such as MY, feed intake, FE, and subsequent CO_2 eq. of milk production.

3.4: Aim and objectives

This study aimed to assess the effect of atypical cycle length (ACL) compared with typical cycle length (TCL) in high-yielding dairy cows on fertility-related key performance indicators (KPIs), MY, FE, and CO_2-eq. production related to extended CI and increased RR. The objectives of the study were to assess the occurrence of ACLs in high-yielding cows and assess the effect of ACL on the CI, PR, and related RR, to assess the effect of CI and RR, in relation to the FE of high yielding cows on the potential CO_2-eq. per kg of component correct MY.

3.5: Materials and methods

3.5.1: Study location and ethical approval

This study was carried out between October 2018 and May 2019 at the University of Nottingham (**UoN**) Centre of Dairy Science and Innovation (CDSI) (MY of 11,300 L/cow/yr.), Sutton Bonington, Leicestershire, UK in accordance with and the prior approval of the UoN Animal Ethics Committee and authority of the UK Animal (Scientific Procedures) Act 1986.

3.5.2: Experimental design

The study was a randomised block design using 49 Holstein-Friesian dairy cattle of a mean MY 11,500 (± 961.5), parity of 2.88 (± 0.27 range 1 to 8), live weight (**LW**) of 738.2 (± 25.13) kg and BCS of 3.1 (± 0.22) at parturition that were selected randomly according to calving date from a 250-cow dairy herd that calved all year round. Animal were allocated according to previous MY, parity, LW and BCSP to receive a PMR with (+Y) and with no yeast (NY) (Actisaf, Phileo by Lesaffre, France) (Fig 3.1) and from parturition onwards these cows had milk samples collected to assess milk progesterone concentrations assessed in accordance with Experiment 1 and were assigned retrospectively according to pp luteal cycle length into blocks that had atypical (ACL) and typical cycle (TCL) lengths using milk P4 concentrations as detailed in Experiment 1 and the diet was found to have no effect on the proportion of cows and MY, parity, LW and BCS at parturition (Fig 3.1) of cows that had ACL and TCL.

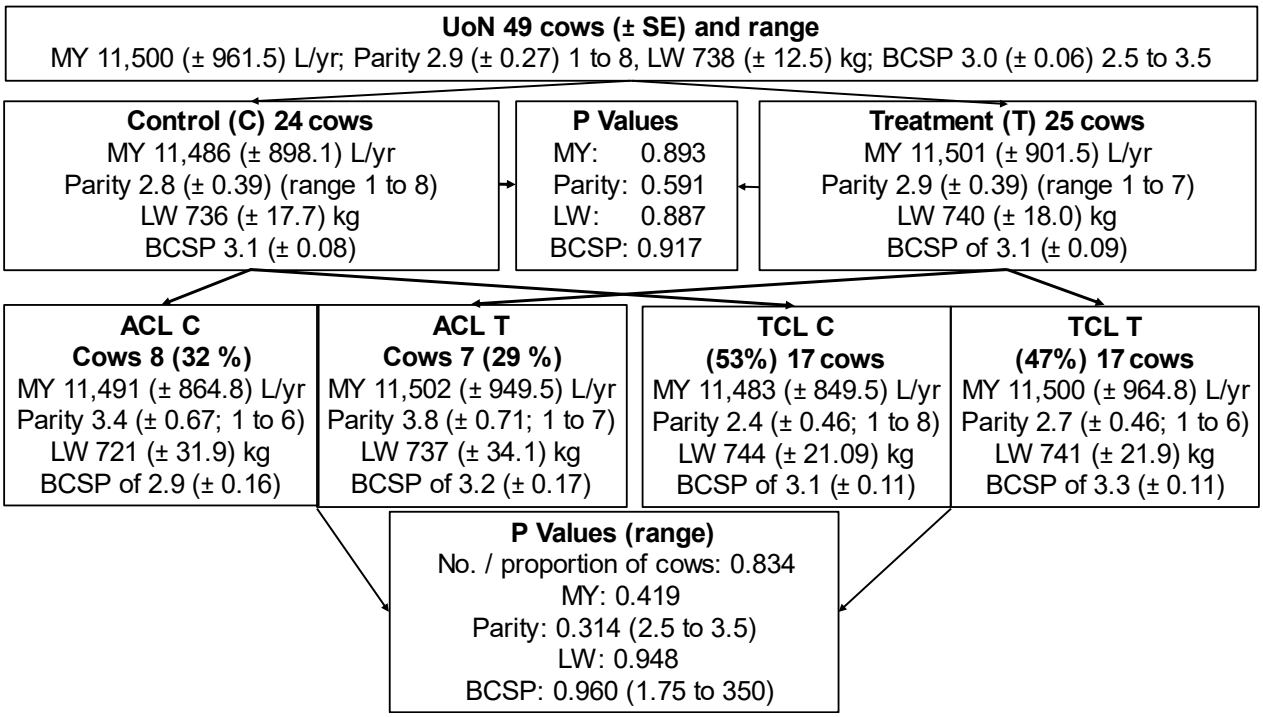


Fig 3.1: Schematic of the proportion of cows that had atypical (ACL) and typical (TCL) cycle lengths from cows that were offered no yeast (NY) and yeast (+Y) in the partial mixed ration

3.5.3: Animal management

The cows were housed in the same group within the same that had 55 free stalls that were fitted with cantilever divisions and deep litter sand beds, which were cleaned daily, and fresh sand was replenished weekly. The feeding alleys were fitted with concrete slats, which were cleaned at 6 h intervals using an automatic above-ground robot (Lely UK Ltd., St Neots, UK) and under-floor scrapers that ran on a 4 h cycle over each 24 h period.

3.5.4: Diets and animal feeding

The cows were offered *adlibitum* access to clean fresh water and a partial mixed ration (**PMR**) that was formulated to supply adequate nutrients for the maintenance requirements, plus 30 L of milk production per day (Table 3.1).

Table 3.1: Partial mixed ration ingredients and composition of mixed ration and pelleted concentrate offered at milking

Ingredients (kg DM/d/cow)	Partial mixed ration	Pelleted concentrate
Diet components		
Maize silage, g/kg DM	275	-
Grass silage, g/kg DM	229	-
Whole crop silage, g/kg DM	231	-
Wheat straw, g/kg DM	9	-
Soya: Rape seed meal, g/kg DM	181	-
Molasses, g/kg DM	27	-
Protected fat, g/kg DM	19	-
Dried distillers grains with solubles	7	-
Minerals and vitamins, g/kg DM[1]	22	-
Total	1000	-
Diet composition		
Dry matter, %	45.2	86.4
CP, % DM	16.8	18.0
ME, MJ/Kg DM	11.8	12.5
NDF, % DM	35.5	21.7
Starch and sugar, % DM	26.2	23.8
Oil, % DM	4.5	4.3
Ash, % DM	7.5	5.6
CO_2-eq. / kg, kg	0.34	1.32

[1]-Dietary minerals and vitamins; calcium, 18%; phosphorus, 10%; magnesium, 5%; salt, 17%; copper, 2,000 mg/kg; manganese, 5,000 mg/kg; cobalt, 100 mg/kg; zinc, 6,000 mg/kg; iodine, 500 mg/kg; selenium, 25 mg/kg; vitamin A, 400,000 IU/kg; vitamin D3, 80,000 IU/kg; and vitamin E, 1,000 mg/kg.

The PMR was offered as a daily feed allowance of 110% of PMR intake. The PMR was replenished once daily at approx. 07.30 hrs in electronic feed intake stations that were fitted with a feed weighing system (Hokofarm Group B.V., Marknesse, Netherlands), which

measured individual animal feed intake on a constant basis. During milking, cows were offered a pelleted dairy compound feed through the robotic milking station in an integral feed bin (Lely Astronaut A3; Lely UK Ltd., St Neots, UK) and this was distributed at a rate of 2 kg/hd/d at parturition, which increased at 0.2 kg/d up to a maximum of 12 kg /hd/d up to 60 d pp, followed by which allocation remained the same until 100 d pp and then was reduced according to individual animal MY and was offered at 0.4 kg/L on a daily basis.

3.6: Measurements

3.6.1: Feed samples and feed analysis

Feed sample collection and analysis was as stated in Experiment 1 (Table 3.1).

3.6.2: Dry matter intake

Individual animal fresh matter (**FM**) PMR intake was measured using automated electronic feed gates and bins (Hokofarm Group B.V., Marknesse, Netherlands) to record the daily PMR intake, which was used to calculate a weekly mean FM intake and the DM contents (Table 3.1) were used to calculate the individual weekly DMI. The pelleted compound (**PC**) feed that was distributed to each individual cows was recorded by the Lely milking robot (Lely Astronaut A3; Lely UK Ltd., St Neots, UK) and used along with the DM contents (Table 1) of the PC to calculate the weekly mean kg of PC DM intake/d. The daily mean PMR and PC DM intake were used to calculate the total daily DMI for each animal.

3.6.3: Milk yield and progesterone concentrations

Individual animal daily milk yield and biweekly composition were measured using the Lely milking robot (Lely Astronaut A3; Lely UK Ltd., St Neots, UK) by collecting 20 ml composite milk samples from individual animals over a 24 h period on two consecutive days (pm and am), which were preserved using a broad-spectrum Microtabs®II (containing Bronopol and Natamycin; D&F Control Systems, Inc., USA) and refrigerated until analysed for milk fat, protein, somatic cell count (**SCC**) and urea concentrations using near infra-red analysis in accordance with AOAC (1990) reference method No. 972.16 (National Milk Records Laboratory, Harrogate, Yorkshire, United Kingdom). Milk yield was recorded in kg/d and weekly milk yield and bi-weekly milk composition were used to calculate fat corrected to 40 g/kg, while energy corrected (EC) yield was calculated as, kg = 0.327 x MY kg + 12.95 x milk fat kg + 7.21 x milk protein kg and reported in kg/d according to Sjaunja et al. (1990) and fat and protein corrected milk *(*FPCM) yield was calculated according to milk kg × ((0.1226 × Fat %) + (0.0776 × Protein %) + 0.2534) (Gerber et al. 2010) and milk solids (kg/d) were calculated as the total of milk fat (kg/d) plus milk protein and were reported in kg/d.

Milk samples (20 ml) were collected and analysed personally as described in Experiment 1 thrice weekly (am) from individual cows from 14 up to 130 (± 0.3) d pp in May 2019. Milk samples were had a broad spectrum Microtabs®II (containing Bronopol and Natamycin; D&F Control Systems, Inc., USA) added and were frozen -22 ° C until they were thawed for 24 h prior to the determination of progesterone concentration using an enzyme-linked immunosorbent assay (**ELISA**) (Ridgeway Science Ltd., Alvingdon, UK) in accordance with Bedere et al. (2016) and the coefficients of variation between assays for ELISA on 5 ng/mL control samples ranged between 8 and 10%. The luteal activity was determined using two consecutive milk progesterone (**P4**) concentration thresholds according to Petersson et al. (2006) as adopted by Cutullic et al. (2011) to establish baseline progesterone concentrations and distinguish between low P4 luteal phase concentration using negative thresholds < 5 ng, positive thresholds > 5 ng and intermediate thresholds = 5 ng, while increases in P4 concentrations between consecutive milk samples was considered to be due to CL activity and decreased P4 concentrations indicated luteolysis of the CL and used distinguishing luteal phases from intergluteal phases. These P4 concentrations were used to calculate the PPI to first P4 rise, PPI to first ovulation, inter-ovulatory luteal cycle length, and PPI to conception according to Cutullic et al. (2011).

3.6.4: Live weight and body condition score

The individual animal LW was measured daily using load cells that were fitted to the base of the Lely robotic milking station platform (Lely Astronaut A3; Lely UK Ltd., St Neots, UK), which were used to calculate a weekly mean LW and used to calculate live weight and liveweight change. Individual animal body condition score (**BCS**) was assessed by the same person throughout the study on a weekly basis using a scoring scale of 1 (thin) to 5 (fat), which was assessed to the nearest quarter point according to AHDB (2014) on a weekly basis and used to calculate the change in pp BCS and report the BCS at parturition (BCSP) and report LW and BCS at 0, 60 and 120 d pp and the change between 0 to 60 d, 60 and 120 d pp and 0 and 120 d pp.

3.6.5: Carbon equivalent impact of infertility

The GHG emissions of the production of the individual feeds used for the dairy cows were taken from the FeedPrint tool (Vellinga et al*.* 2013) and used to calculate the kg of CO2 eq. for the PMR and pelleted compound concentrate offered in the robot to calculate the CO_2-eq of the diet. The CO_2-eq. for additional days CI and % RR were calculated as previously stated in Experiment 1.

3.5.6: Animal health

Herd records were used to assess animal health and one cow was removed from the study data and euthanized due to stomach ulceration, which was deemed unrelated to the research.

3.5.7: Statistical analysis

Data was collated in Microsoft Office Excel 2016 and used to calculate weekly mean values for MY, milk fat, protein, lactose, SCC and urea, LW, DMI, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR and PPI to conception and CI. The mean weekly data for MY, corrected MY, milk fat, protein, lactose, SCC and urea, BCS, LW, DMI, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR and PPI to conception and CI were assessed using the Anderson-Darling test for normality of distribution using Minitab statistical software (Version 17.2.1, 2016, State College, PA) and found to be normally distributed except for the milk SCC, which was transformed using the $\log^{base10}$, prior to analysis. The effect of previous diet treatment on the number/proportion of cows, previous milk yield, parity, BCS and LW of cows that had ACL and TCL was assessed using the general linear model (GLM) ANOVA command by each variable/ response using animal as a random effect and previous diet and ACL and TCL as fixed effects in the model. The effect of ACL and TCL were assessed mean BCS was assessed using regression analysis, using BCS as categorical data and ACL and TCL as fixed effect and animal as a random effect in the model. The weekly mean milk yield, composition, FCM, ECM, FPCM, total protein and fat, and milk solids (MS) and lactose, feed intake, BCS, feed efficiency, milking frequency, animal activity and calculated CO_2 eq. were analysed using the General Linear Mixed Model (GLM) command in ANOVA by each variable/ response using animal as a random effect and ACL and TCL as fixed effects in the model. The number of cows that had ACL and TCL, conception rate and the number of inseminations were assessed using Chi-square test, using ACL and TCL as row and response as a column in the table. Data were reported as means (± SE) and range with P values and the existence of significant differences between means were reported at a $P<0.05$ and tendencies at $P<0.10$.

3.7: Results

3.7.1: Reproductive performance

In this study ACL occurred in 31 % of the dairy cows and ACLs were 29 d longer and the PPI to first P4 rise was 6.9 d longer compared with cows that had TCL (Table 3.2). While there was no difference in the PPI to first AI, CR, PR, PPI to conception and number of AI per

conception by 130 d pp, cows that had ACL had a final PPI to first AI that was 17.9 d longer, PPI to conception that as 49.3 d longer and a CI that was 55 d longer than cows that had TCL. The final CR and PR did not differ between cows that had ACL and TCLs, however 6% of cows that had TCLs did not conceive and survive, which increased the number of replacements required due to infertility.

Table 3.2: Mean reproduction performance (± se) and number of dairy cows that had atypical and typical postpartum oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Observations, No. cows	15	34	-
Mean cycle length	50.9 (4.19)	21.5 (2.78)	<0.001
PPI to first pp P4 rise, d	28.7 (1.85)	21.8 (1.23)	0.003
Fertility up to 130 d pp			
PPI to first AI, d	59.7 (3.39)	52.8 (5.41)	0.285
Conception rate, %	47	68	0.168
PPI to conception, d	83.1 (9.93)	71.2 (5.48)	0.165
AI per conception, No.	2.0	2.1	0.859
Pregnancy rate (PR), %	47 (12.6)	68	0.165
Calving interval (CI)[1], d	366 (9.93)	354 (5.48)	0.300
Fertility results up to 160 d pp			
PPI to first AI, d	70.7 (6.43)	52.8 (4.34)	0.026
Conception rate, %	100	94	0.348
PPI to conception, d	147.4 (15.10)	98.1 (10.30)	0.010
Pregnancy rate (PR), %	100 (5.20)	94 (3.40)	0.348
Calving interval (CI), d[1]	430 (15.10)	375 (10.30)	0.005

[1] – Calving interval (CI) – Calculated from PPI to conception + 283 d gestation. [2] – Replacement rate calculated on an assumed replacement rate of 20% and CI 365 d. [3] – Combined replacement rate for additional days of calving interval + 100 % for replacements.

3.7.2: Milk yield, composition, milking frequency and parity

The cows that had ACL were of greater parity and had higher milk SCC, and produced greater MY, FC, ECM, FPCM, milk fat, protein and solids yields, despite having lower milk fat and protein concentrations compared with cows that had TCL up to 130 d pp (Table 3.3). The milk

lactose and urea concentrations and voluntary milking frequency did not differ between cows that had ACL and TCL during the first 130 d pp.

Table 3.3: Mean yield and composition (± se) of milk of dairy cows that had atypical and typical postpartum oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Milk yield, kg/d	51.0 (0.52)	47.6 (0.35)	<0.001
FC milk yield, kg/d [1]	47.9 (0.56)	46.5 (0.37)	0.037
ECM yield, kg/d [2]	50.5 (0.48)	48.3 (0.32)	<0.001
FPCM yield, kg/d [3]	48.9 (0.46)	46.8 (0.31)	<0.001
Milk fat, g/kg	37.8 (0.39)	39.6 (0.26)	<0.001
Milk protein, g/kg	31.7 (0.16)	32.5 (0.11)	<0.001
Lactose g/kg	46.9 (0.08)	47.9 (0.05)	0.583
Urea, %	0.28 (0.005)	0.27 (0.003)	0.281
Milk fat, kg/d	1.92 (0.022)	1.86 (0.015)	0.036
Milk protein, kg/d	1.61 (0.015)	1.53 (0.010)	<0.001
Milk solids, kg/d	3.52 (0.033)	3.39 (0.022)	0.001
Log^{base10} SCC, cells/ml	1.61 (0.030)	1.53 (0.020)	0.021
Parity, No.	3.60 (0.104)	2.56 (0.07)	<0.001
Milking frequency, No./d	2.97 (0.041)	2.90 (0.037)	0.165

[1] – FC – Fat corrected yield, kg = Milk yield kg x milk fat kg / 40.0. [2] – EC – Energy corrected yield, kg = 0.327 x milk yield kg + 12.95 x milk fat kg + 7.21 x milk protein kg (Sjaunja et al. 1990). [3] – FPCM = Milk kg × ((0.1226 × Fat %) + (0.0776 × Protein %) + 0.2534) (Gerber et al. 2010).

3.7.3: Dry matter intake and feed efficiency

The total DMI tended to be greater and the pelleted concentrate intake, CO_2-eq. from pelleted concentrate and activity levels were greater in cows that had ACL compared with TCLs (Table 3.4). The PMR intake, CO_2-eq. from PMR, and time spent on rumination did not differ between cows that had ACL and TCL. In terms of FE, cows that had ACL had greater EC, FPCM and milk protein production levels per kg of DMI and total DMI per day was lower per kg of EC, FPCM and milk protein compared with cows that had TCL. The CO_2-eq. total diet DMI per kg of ECM, FPCM and milk protein yield did not differ between cows that had ACL and TCL.

Table 3.4: Mean dry matter intake (DMI) (± se), rumination and feed efficiency of dairy cows that had atypical and typical postpartum oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Total dry matter intake (DMI), kg/d	24.8 (0.23)	24.3 (0.15)	0.096
Partial mixed ration intake, kg DM/d	17.7 (0.20)	17.5 (0.13)	0.545
Pelleted concentrate intake, kg DM/d	9.4 (0.13)	8.9 (0.08)	0.001
CO_2-eq. partial mixed ration, kg DM	2.7 (0.03)	2.6 (0.02)	0.493
CO_2-eq. pelleted concentrate, kg DM	9.4 (0.13)	8.9 (0.08)	0.001
CO_2-eq. total DMI, kg DM	12.1 (0.13)	11.6 (0.09)	0.001
Rumination, min/d	466 (2.60)	467 (1.69)	0.671
Activity, steps per day	593 (6.02)	558 (3.91)	<0.001
Feed efficiency			
EC yield[1] / kg total DMI, kg	2.07 (0.022)	2.01 (0.014)	0.037
FPCM yield [2] / kg total DMI, kg	2.00 (0.021)	1.95 (0.014)	0.035
Milk protein/kg DMI, g	65.7 (0.59)	63.7 (0.39)	0.005
Total DMI / kg ECM, kg	0.50 (0.005)	0.51 (0.003)	0.016
Total DMI / kg FPCM, kg	0.51 (0.005)	0.53 (0.003)	0.015
Total DMI / kg milk protein, kg	15.5 (0.134)	16.0 (0.09)	0.002

[1] – EC – Energy corrected yield, kg = 0.327 x milk yield kg + 12.95 x milk fat kg + 7.21 x milk protein kg (Sjaunja et al. 1990). [2] – FPCM = *Milk* kg × ((0.1226 × Fat %) + (0.0776 × Protein %) + 0.2534) (Gerber et al. 2010).

3.7.4: Body condition score and live weight

The mean LW and BCS and range in BCS did not differ at 0, 60, and 120 d pp and pp change in LW and BCS between 0 and 60, 60 and 120, and 0 and 120 d pp between cows that has ACL and TCLs (Table 3.5).

Table 3.5: Mean and range in body condition score BCS (± se) of dairy cows atypical and typical postpartum oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Range in body condition [1]			
At parturition, 0 d pp	2.50 to 4.20	3.10 to 3.75	0.653
At 60 d pp	2.00 to 3.75	2.25 to 4.00	0.301
At 120 d pp	1.75 to 4.25	2.0 to 4.25	0.389
Mean body condition [1]			
At parturition, 0 d pp	3.00 (± 0.114)	3.10 (± 0.076)	0.652
At 60 d pp	2.83 (± 0.126)	2.99 (± 0.076)	0.229
At 120 d pp	2.83 (± 0.155)	2.99 (± 0.103)	0.397
Change in mean body condition			
0 to 60 d pp	-0.20 (± 0.091)	-0.10 (± 0.060)	0.377
60 to 120 d pp	0.00 (± 0.082)	-0.00 (± 0.055)	0.971
0 to 120 d pp	-0.20 (± 0.119)	-0.10 (± 0.079)	0.501

[1] – Body condition: Assessed (0 to 5 points scale, with 0.25-point intervals) according to AHDB, 2021

Table 3.6: Mean of live weight (± se) of dairy cows atypical and typical postpartum oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Live weight, kg			
At parturition, 0 d pp	729 (± 22.9)	742 (± 15.2)	0.625
At 60 d pp	724 (± 21.6)	742 (±14.4)	0.491
At 120 d pp	742 (± 21.1)	761 (± 14.0)	0.459
Liveweight change, kg			
0 to 60 d pp	-5.2 (6.63)	-0.71 (4.20)	0.576
60 to 120 d pp	18.4 (4.92)	19.4 (3.20)	0.872
0 to 120 d pp	13.2 (9.79)	18.7 (6.50)	0.645

3.7.5: Carbon equivalent related to infertility

The number of days over a 365 CI and RR for addition days over a 365 d CI and infertility related replacements and infertility related CO_2-eq. at < 130 d pp did not differ between cows

that had ACLs and TCLs (Table 3.6), while at 160 d pp the number of days over a 365 d CI and RR required due to the greater CI and CO_2-eq. related to the days over a 365 CI was greater for cows that had ACLs than TCLs. The RR and infertility-related replacements and CO_2-eq. did not differ between cows that had ACLs and TCLs.

Table 3.7: Carbon dioxide equivalent (± se) of dairy cows that had atypical and typical postpartum oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
At 130 d pp:			
Days over 365 d calving interval (CI), d	1.1 (38.90)	-60 (21.5)	0.180
Replacement rate for additional CI, %	0.06 (0.544)	-0.59 (0.300)	0.301
Replacement rate, %	53 (12.6)	32 (8.4)	0.172
Infertility related replacements, %	54 (15.2)	31 (6.44)	0.156
CO_2-eq. for days over 365 d, kg	17 (580)	-895 (320)	0.180
CO_2-eq. for herd replacements CI, kg	2597 (613.0)	1576 (407.0)	0.172
CO_2-eq for infertility, kg	2599 (616.0)	1556 (409.0)	0.165
At 160 d pp:			
Days over 365 d calving interval (CI), d	65.4 (15.1)	16.1 (10.3)	0.010
Replacement rate for additional CI, %	3.6 (0.83)	0.9 (0.57)	0.010
Replacement rate for empty rate, %	0.0 (5.17)	5.9 (3.43)	0.348
Infertility-related replacements, %	3.6 (5.19)	6.7 (3.44)	0.618
CO_2-eq. for days over 365 d, kg	974 (225.0)	240 (154.0)	0.010
CO_2-eq. for herd replacements, kg	0 (252.0)	286 (167.0)	0.348
CO_2-eq for infertility 365 CI, kg	974 (325.0)	512 (216.0)	0.242

[1] – Calculated from an assumed RR of 20% and CI of 365 d [2] – Combined CO_2-eq for additional herd RR and CI greater than 365 and 375 d

3.8: Discussion

3.8.1: Cycle length and fertility

In this study a large proportion of high-yielding cows exhibited prolonged ACL (Table 3.2), which was in keeping with previous studies that equally found that approx. 40% of dairy cows exhibit ACLs (Petersson et al. 2006; Windig et al. 2008; Cutullic et al. 2011). Moreover, other studies of Holstein Friesian (**HF**) cows have shown that this breed in particular exhibits more

ACLs than other dairy breeds, such as Jersey (Petersson et al. 2006; Cutullic et al. 2011; Piccand et al. 2013). The PPI to first pp P4 rise was longer in cows exhibiting ACLs compared with cows exhibiting TCLs, and such delays in the commencement of pp luteal activity have been shown to be the most common form of ACL, accounting for between 13 and 30% of the variance in PPI to commencement of ovarian activity (Petersson et al. 2006; Windig et al. 2008; Cutullic et al. 2011). This has been related to individual animal genetics and negatively associated with greater MY (Veerkamp et al. 2000; Royal et al. 2002b; Petersson et al. 2007). In this study, the cows that exhibited ACLs did have greater MY than cows exhibiting TCLs. The PPI to conception and CI was longer for cows that had ACL, however, assuming that ACL is directly related to MY would be too simplistic. The exhibition of ACLs is complex, involving a combination of factors that include the deleterious effect of higher genetic merit for MY (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and while the occurrence of prolonged luteal phases have been related to MY (Royal et al. 2002b; Kafi et al. 2012) it may be mediated through other biological factors such as NEB and pp loss of BCS (Burke and Roche, 2007; Chagas et al. 2008), which affect other fertility-related factors.

3.8.2: Milk yield

The occurrence of prolonged luteal phase has been directly related to increases in the individual milk yield of dairy cows (Royal et al. 2002b; Kafi et al. 2012) and the cows in this study that had ACL produced greater amounts of MY, FC, ECM, FPCM, milk fat, protein and solids yields, despite having a lower milk fat and protein concentrations compared with cows that had TCL (Table 3.3). Milk lactose and urea concentrations and voluntary milking frequency did not differ between cows that had ACL and TCL. Greater MYs can increase the amount of nutrients partitioned towards MY and extend this beyond early lactation (Leroy et al. 2010a), which has been defined in fertility terms as < 70 d pp (Chiba, 2009) and result in this coinciding with first insemination, which may lower CR and PR. These, and the PPI to the first insemination, are essential in achieving a regular calving CI that is associated with greater and more efficient milk production (Royal et al. 2008). Increases in the CI and lower PR increases RR, due to the association between greater CI and lower survival rates. Equally extending the mating period and increasing the CI to achieve higher PR needs to be considered in combination with FE, especially given the negative effect of RR on CO_2 eq. and milk production costs to summarize sustainable approaches to dairy food security.

Increasing the level of MY of individual cows has often been mentioned as an option to mitigate CH_4 emission intensity (CH_4 emissions per unit of milk produced) due to the dilution of maintenance (Capper and Bauman, 2013), and increasing FE (VandeHaar and St-Pierre, 2006) and the cows in this study that exhibited ACLs did yield greater amounts of milk and

were more FE in terms of ECM, FPCM, and milk protein production. However, this approach neglects the effect of high MY on fertility, which has often been associated with a lower PR (Britto et al. 2021) and extended CI (Pryce et al. 1997; Inchaisri et al. 2010), but more importantly RR (Bell and Wilson, 2018), however, most studies fail to evaluate the effect of MY on FE, along with the effect of CI and PR on RR. The cows that exhibited ACLs in this study had greater MYs and while they did not have lower the CR and PR at > 130 d pp and final CR and PR, the increased in the PPI to conception and CI increased the number of herd replacement rate (RR) compared with those that had TCLs. In most countries, dairy herds contain a large proportion of primiparous cows and RR range from 20 to 40% (Le Cozler et al. 2008).

3.8.3: Body condition and live weight

In this study, the BCSP of cows that had TCL was within industry-recommended BCSP targets (AHDB, 2020) and higher-yielding dairy cows that have greater metabolic demands (Markusfeld, Ezra and Galon, 1997) typically mobilize more BCS following parturtion to support MY due greater metabolic limitation of pp DMI (Drackley and Reynolds, 2021; Margerison, 2022). Cows in higher BCS can experience lower DMI and greater NEB compared with cows in optimum BCSP at parturition (Gwazdauskas, Wilcox and Thatcher, 2010), however BCS was within industry standards, DMI and BCS at parturition and pp change in BCS was not greater in cows that had TCL than ACL. Cows that had ACL had greater MY in this study and higher yielding cows tend to mobilize more BCS pp to support milk production, which can be detrimental to the health and fertility of the cow (Pryce et al. 1999) and its subsequent lifespan (Bell et al. 2010). However, there was no difference in pp loss of BCS between cows that had ACL and TCL (Table 3.7) in this study. Despite this, cows that had ACL had an extended PPI to first P4 rise, the PPI to conception and CI. The pp change in BCS was marginal and well below industry (-0.5 BCS) recommendations (AHDB, 2020) in this study and dairy cows that lose < 0.5 pp BCS should return to ovarian activity and first ovulation within 30 d pp (Pryce et al. 2001; Garnsworthy, 2006; Patton et al. 2007), which was the case in this study in cows that had TCL and ACL. In this study, it is unlikely that BCS had any negative effects on ovarian activity (Diskin et al. 2003; Friggens and Chagunda, 2005; Chagas et al. 2007; Leroy, et al. 2008; Aguilar-Perez et al. 2009) and involved in extending the PPI to conception (Domecq et al. 1997; Lopez-Gatius et al. 2003; Mäntysaari et al. 2022), lowering of CR (Butler, 2005), increased infertility (Westwood et al. 2002; Buckley et al. 2003) and/or was inadequate nutrient supply having a negative effect on follicular growth, conception and pregnancy recognition (Murphy et al. 1991; Diskin et al. 2003; McDougall et al. 2005; Aguilar-Perez et al. 2009).

The achievement of recommended BCSP and limited mobilization of BCS during early lactation can lower the occurrence of ACL (Cutullic et al. 2012), however despite this the cows in this study returning to pp ovarian activity and first ovulation within 30 d pp, of these cows that had ACL despite having pp BCS loss < 0.5 BCS during early lactation (< 70 d pp) they had longer PPI to conception and CI that has previously been associated with BCS lose > 0.5 (Pryce et al. 2001; Garnsworthy, 2006; Patton et al. 2007) indicating that the final PPI to first AI was longer and PPI to conception and CI were longer for cows that had ACLs than those that had TCLs. This was unlikely to be due to the effect of BCSP, BCS loss and NEB on the onset of ovarian activity, oocyte quality and CR due to endocrine regulation of changing lipogenesis to lipolysis during early lactation (Garcia-Carcia, 2012) and other factors that affect this need to be evaluated.

3.8.4: Carbon dioxide equivalents related to calving interval and herd replacement rate

In this study greater herd RR, due to lower PR and lower survival rates of cows that had ACL, while these cows had greater MY and were more FE than cows that had TCL. The feed related CO_2-eq. emissions / kg FC, EC, FPCM and milk protein did not differ and reduction in CO_2-eq. emissions would need to come from less CO_2-eq. emissions related to maintaining and milking fewer cows and producing less CO_2-eq. emissions related to manure storage, fixed costs and herd replacements. Even one percent increase in PR and survival rate of cows greatly reduces CO_2-eq. emissions per cow (−48 to −53 kg) and per unit milk solids (−91 to −98 g/kg) (Bell and Wilson, 2018). While a unit reduction in enteric CH_4 would also notably reduce CO_2-eq. emissions per cow (−48 to −50 kg) and per unit milk solids (−75 to −79 g/kg) should the selection for lower emission cows be possible commercially (Bell and Wilson, 2018). This would require the evaluation of the effect of increased MY on FE, CI and PR and replacement rate combined.

This study showed that (Table 3.11) cows that had ACL had an extended 365 d CI and additional herd replacements were required due to this greater CI and increased the kg of CO_2-eq. compared with TCLs. Over the last decades increasing the MY of individual cows has been shown to reduce the length of productive life (LPL) markedly (Knaus, 2009) and increasing the LPL, lowers the RR by achieving a CI closer to 365 d (Bell and Wilson, 2018) along with increasing the PR, could be considered an option to mitigate and reduce GHG emissions from the rearing of replacement animals (Zehetmeier *et al.* 2014; Bell *et al.* 2015). Previous studies have shown that herd replacement rate has the greatest environmental impact on dairy systems and the ability to reduce CO_2-eq. emissions per cow (−48 to −53 kg) and per unit milk solids (−91 to −98 g/kg) per one percent increase in RR (Bell and Wilson, 2018), which in this study could be achieved by lowering the CI of cows with ACL. In this study,

at 160 d pp cows that had ACLs did not increase CO_2-eq. related to herd replacements compared with cows that had TCLs, however RR related to infertility were higher at 130 d pp in cows that had ACLs and TCLs, all of which were well beyond the 85 d pp (Grimard et al. 2006) required to achieve a 365 d and/or even a 375 d CI. This study considered the combined effects of increased MY on FE, CI and RR and showed that the evaluation of the effect of CI and PR combined are factors that warrant further evaluation along with empty rate and FE in a larger scale study.

3.9: Conclusions

Dairy cows that exhibited ACL in this study had greater FC, EC, FPCM, milk protein, fat and MS yields and were more FE in the production of EC, EPCM and milk protein yields. However, ACL were not related to poor BCS at parturition and greater pp loss of BCS. Cows that had ACL had an extended CI and PR was low at 130 d pp, which increased CO_2 eq. related to infertility substantially in higher MY cows that were more FE. However, this study was limited by the number of cows involved and was unlikely to result in significant correlations between factors that may be related to ACL and indicate that this approach needed to be applied using larger data sets.

3.10: Further work

This study showed that ACL was relatively common in high milk yielding FE dairy cows and that ACL substantially increased CO_2-eq., which in this study was due to RR at 130 d pp and an extended CI at 160 d pp rather than RR, which warranted further evaluation in a larger study, which require individual feed intake measuring facilities to calculate FE, that potentially needed to come from differing sites, to evaluate the correlation between ACL and MY and FE, and CI and PR related RR and the potential effect on CO_2-eq. emissions from milk production.

Appendix 3: Chi-Square tables

Table: Chi-Square Rows: Cycle length Columns: Conception / Pregnancy rate < 130 d

	0	1	All
Atypical	8	7	15
	5.816	9.184	
Normal	11	23	34
	13.184	20.816	
All	19	30	49

Cell Contents: Count Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	1.930	1	0.165
Likelihood Ratio	1.904	1	0.168

Table: Chi-Square Rows: Cycle length Columns: Conception / Pregnancy rate < 160 d

	0	1	All
Atypical	0	15	15
	0.612	14.388	
Normal	2	32	34
	1.388	32.612	
All	2	47	49

Cell Contents: Count Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.740	1	0.348
Likelihood Ratio	0.734	1	0.342

Chapter 4.0

Experiment 3.0

Relationship between ovarian cycle length and milk yield, feed efficiency, calving interval, replacement rate, and potential carbon emissions from milk production

Relationship between ovarian cycle length and fertility, milk yield, feed efficiency, calving interval, replacement rate, and potential carbon emissions from milk production

4.1: Abstract

Increasing the milk yield (**MY**) and feed efficiency of dairy cows is essential in lowering the environmental impact and financial cost of milk production and the achievement of net zero carbon. However, few studies have collected larger sets of detailed biological data from lactating dairy cows to assess the relationship between feed efficiency, fertility, and carbon emissions (CO_2 eq.). This study aimed to evaluate the occurrence of atypical ovarian cycles (**ACL**) and the relationship with fertility, energy (**ECM**), fat and protein corrected (**FPCM**) and milk protein yield, feed efficiency (**FE**), and CO_2 eq. related to CI and RR using 88 dairy cows that were allocated postpartum (pp) into groups of cows that had ACL and typical cycle length (**TCL**). ACL was relatively common occurring in 30% of cows and increased ovarian cycle length (P=0.047), postpartum interval (**PPI**) to first milk progesterone (**P4**) rise (P<0.001), PPI to the first insemination (P=0.006), and the number of inseminations per conception (P=0.002), but had no effect on the CR to first service, PPI to conception, pregnancy rate (**PR**) and calving interval (**CI**). Moderate correlations existed between ovarian cycle length and the PPI to the first AI, the no. of inseminations/conception, and conception rate. There was no difference in MY, and FPCM yields, BCS or LW, and pp change in BCS and LW, DMI, FE, RR, and CO2-eq. related to RR, CI and infertility between cows that had ACL and TCL. Correlation analysis showed low strength in the relationship between ovarian cycle length and MY, FE and CI or RR. However, cows produced substantial amounts of CO_2-eq. due to an extended CI and RR, which was highly correlated with RR. It was concluded that the occurrence of ACL was relatively common, delaying the PPI first P4 rise and insemination and increasing the number of inseminations per conception, which was not related to greater MY, pp BCS loss and FE. However dairy cows in this study had substantial amounts of CO_2 eq. attributed to an extended CI and a greater RR.

Keywords: Ovarian cyclicity, Feed efficiency, Calving interval, Replacement rate, Carbon dioxide emissions

4.2: Introduction

Increasing the milk yield (**MY**) of individual cows has successfully achieved greater feed efficiency (**FE**) required to dilute the nutrient costs related to animal maintenance and milk production (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014). While this is essential in maintaining global warming < 1.5°C up to 2030 and achieving net zero emissions by 2050 (UN, 2022), increasing

the MY of dairy cows can have a deleterious effect on the regularity of ovarian cyclicity (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and extend the calving interval (**CI**) (Experiment 2) (Pryce et al. 1997; Inchaisri et al. 2010). Moreover lower pregnancy rate (**PR**) (Brito, 2021) increased herd replacement rate (**RR**) and CO_2 eq. related to CI and RR (Experiment 1 and 2), which are considered to be an optimal indicator of the profitability (Bell and Wilson, 2018) of dairy herds. The return to ovarian activity, conception rate and the subsequent postpartum interval (**PPI**) to conception are important parameters that evaluate the reproductive and economic performance of the dairy herd (Harman et al. 1996). Minimising the days open is economically beneficial in increasing milk yield in relation to labour and feed costs (Inchaisri et al. 2010) and in increasing the number of calves born (Cabrera, 2014) and lifetime productive days (González-Recio et al. 2004). An annual calving interval was considered an optimal indicator of fertility and profitability of dairy herds (Pryce et al. 1997; Inchaisri et al. 2010; Bell and Wilson, 2018) and reduced carbon dioxide emissions (**CO_2-eq.)** for milk production related to a greater calving interval (**CI**) (Bell and Wilson, 2018; Experiment 2).

Greater genetic merit for milk yield has been cited to be responsible for 13 to 30% of the observed variance in ovarian activity (Veerkamp et al. 2000; Royal et al. 2002b; Petersson et al. 2007), which is mainly related to a delayed first ovulation (Petersson et al. 2006; Cutullic et al. 2011; Experiment 2). Greater individual cow milk yield has been found to lower pregnancy rates (**PR**) (Brito et al. 2021; Experiment 1), which reduces the productive length of dairy cows (Knaus, 2009) and increases herd culling due to infertility (Pryce et al. 1997; González-Recio et al. 2004; Inchaisri et al. 2010; Cabrera, 2014). This increases breeding costs (González-Recio et al. 2004; Cabrera, 2014) and herd replacement rate (**RR**) (Zehetmeier *et al.* 2014; Bell *et al.* 2015), which increases CO_2-eq. (Experiment 1). This has a substantive negative effect on the financial cost (Yusuf et al. 2011; Horn et al*.* 2012; De Vries, 2017; Bell et al., 2015), lowering the number of calves born (Cabrera, 2014) and lifetime productive days (González-Recio et al. 2004), increasing the CO_2-eq. of dairy production systems (Bell and Wilson, 2018; Experiment 1 and 2). However, there are few published studies have collected adequate amounts of biological data to assess the occurrence of ACL and its relationship with the PPI to conception and CI, PR and RR, and CO_2-eq. related to infertility and the production of milk for human consumption, using larger numbers of cows, a wider range of milk yields, and the inclusion of fat and protein-corrected milk production.

4.3: Aim and objectives

The aim of this study was to evaluate the occurrence of atypical cycles and the relationship with fertility, milk production, and FE and its effect on CO_2-eq. related to CI, RR, and milk

production. The objectives were to assess pp cycle length and occurrence of ACLs and assess the strength of relationship between ACL and the PPI to first P4 rise and conception rate, PR and CI, milk component yield, FE, and CO_2-eq. related to feeding, CI, RR, and fat and protein-corrected milk (FPM) production to evaluate the main factors contributing to the Co2-eq.

4.4: Materials and methods

4.4.1: Study location and ethical approval

This study was completed using data that had been collected previously at the University of Nottingham (UoN) (Leicestershire, UK) and Harper Adams University (HAU) (Shropshire, UK) between April and December 2013. All the animal management, sample collection and research activities were completed under the authority of the United Kingdom Animal (Scientific Procedures) Act 1986 and with the prior approval of the appropriate UoN and HAU animal ethics committees.

4.4.2: Experimental animals

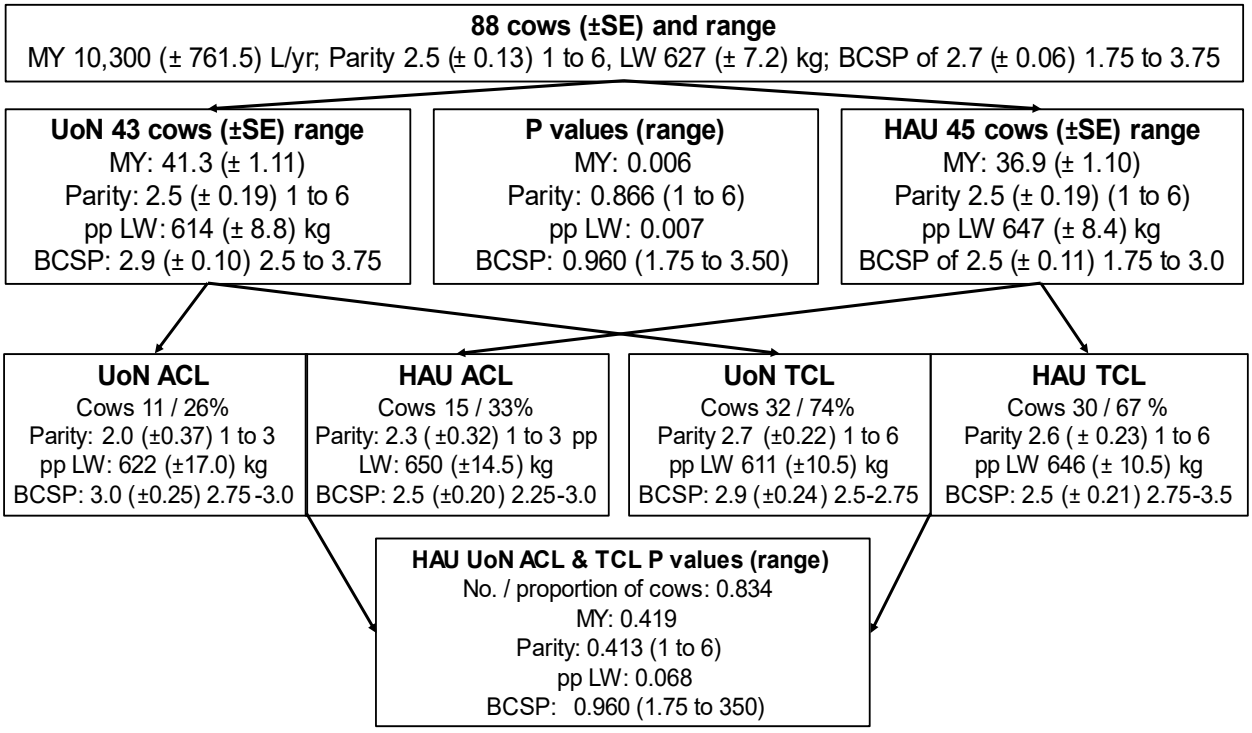


Fig 4.1: Schematic of the proportion of cows that had atypical (ACL) and typical (TCL) cycle lengths from the University of Nottingham (UoN) and Harper Adams University (HAU) experimental sites

This study was carried out using 88 Holstein-Friesian dairy cows (43 at UoN and 45 at HAU) that were selected at random from all year-round calving herds at 10 to 14 d pp (Fig 3.1) and were offered one of three experimental diets that had typical (TPHS) or low dietary crude protein with higher (LPHS) and lower (LPLS) starch concentrations. The animals in this study calved over a 126-d period, had a mean parity of 2.5 (± 0.13) ranging from 1 to 6, LW of 627 (± 7.2) kg and BCS of 2.66 (± 0.38) ranging from 1.75 to 3.75 at parturition and were allocated retrospectively into one of two groups according to the pp exhibition of ACL and TCL as described previously in Experiment 1. The experimental site (P=0.571) and diet (P=0.124) had no effect on the number and or proportion of cows that had ACL and TCL, despite the LW of cows being greater at HAU compared with UoN (Fig 4.1).

4.4.3: Animal housing and management

At UoN and HUA the cows were housed in the same area of a free stall building that was fitted with 5% spare cantilever cubicles, which were fitted with foam filled mats that were cleaned and bedded daily, using sawdust that was replenished and they had lime flour applied weekly. At UoN and HAU the feed and cubicle alleys were fitted with solid concrete floors that were scraped four times and twice daily respectively. At UoN cows were milked automatically up to 3 times daily by a milking robot (A3 Astronaut, Lely UK Ltd, St Neots, UK), while cows at HAU cows were milked twice daily at approx. 06:00 and 16:00 h through a 40-bale rotary parlour (GEA Farm Technologies, Germany) on which concentrates were not offered during milking. All cows had access to fresh clean water ad-libitum.

4.4.4: Diets and animal feeding

The concentrated feed ingredients used in the preparation of the mixed ration and offered at milking were supplied, in the same batches, to UoN and HAU (Phoenix Feeds Ltd, Ramsgreave, Lancashire, UK). At HAU the mixed ration was mixed (Table 4.1a) using a compact forage diet mixer (Keenan, Warwickshire, UK) (± 1 kg) and mix and smart feeder system (Mullerup, Ullerslev, Denmark) at UoN. Individual animal mixed ration fresh matter intake (± 0.1 kg) was measured using an automated feed intake measuring systems at HAU (Insentec (Marknesse, Netherlands) and UoN (Fullwood Ltd, Ellsmere, UK) that were operated using electronic individual animal identification ear mounted tags. The PMRs were offered adlibitum at ≥ approx. 10% daily mixed ration intake and individual samples of pasture and maize silage (≈ 500 g DM) were collected twice weekly, oven dried and used to adjust the ratio of maize:grass silage on a DM basis (Table 4.1a). The cows at UoN had access to supplementary concentrates at a rate of 7.35 kg/d FM, which was limited to a maximum of 3.5 kg at any one visit and distributed by the milking robot (Table 4.1 a and b). At HAU the equivalent feed ingredients were supplied as straights and blends as a total mixed ration.

4.4.5: Feed samples, analysis, carbon equivalents and feed intake

Table 4.1a: Composition of mixed ration and concentrate feeds offered to dairy cows that had atypical and typical oestrus cycles

	Partial mixed ration (kg DM/d/cow)	Pelleted concentrates (g/kg)
Grass silage	11.0	
Maize silage	9.1	
Wheat straw	0.5	
Wheat	-	68
Maize grain	-	68
Sugar beet pulp	-	177
Wheatfeed	-	109
Soya hulls	-	238
Cane molasses	0.12	68
HiPro soya	-	422
Protected soya	1.7	136
Rapeseed meal	2.0	136
Urea	-	16
Rumen protected fats	-	28
Minerals and vitamins [1]	0.22	

[1-] Minerals and vitamins: Calcium, 18%; phosphorus, 10%; magnesium, 5%; salt, 17%; copper, 2,000 mg/kg; manganese, 5,000 mg/kg; cobalt, 100 mg/kg; zinc, 6,000 mg/kg; iodine, 500 mg/kg; selenium, 25 mg/kg; vitamin A, 400,000 IU/kg; vitamin D_3, 80,000 IU/kg; and vitamin E, 1,000 mg/kg (ABN Ltd., Peterborough, UK).

Individual samples (≈ 500 g DM) of the PMR, silages, straw and blended and pelleted concentrates were collected twice weekly and frozen at -20°C. These feed samples feeds were subsequently pooled monthly and analysed (Sciantec Analytical, Cawood, North Yorkshire, UK) for NDF and ADF concentration, determined sequentially according to Van Soest et al. (1991) using a heat-stable α-amylase (Sigma, Gillingham, UK) with the omission of sodium sulphite and starch concentration, which was determined according to method 996.11 (AOAC, 2012). The CP concentration was determined using the DUMAS method 990.03 (AOAC, 2012) using a Leco Truspec Analyser, while water soluble carbohydrates (WSC) were determined according to MAFF (1986) and metabolizable energy (**ME**) was

determined using the calculations detailed by MAFF (1993). The silage analysis was completed using NIR (FOSS, 5000) by applying the appropriate spectra models for maize and grass silages. The individual animal dry matter intake and Co2-eq of the diet were calculated as described in Experiment 2.

Table 4.1b: Nutrient concentrations of mixed ration and concentrate feeds offered to dairy cows that had atypical and typical oestrus cycles

	Mixed ration	Concentrates
Dry matter, %	48.2	86.5
CP, % DM	16.8	18.2
ME, MJ/Kg DM	11.6	12.5
NDF, % DM	35.9	5.1
Starch and sugar, %DM	36.4	38.2
Oil, % DM	4.00	4.70
Co2 eq. / kg, kg[1]	0.35	1.32

[1] - Co2 eq. / kg, kg was calculated using data from Feed Print for each feed source and component in the mixed ration and concentrate component of diet.

4.4.6: Live weight and body condition

At UoN cows were weighed using the milking robot (Experiment 2) and used to calculate an average over a 14-d period, while at HAU cows were weighed manually and LW was reported at 14 d intervals. The BCS was assessed at 14 d intervals, as described in Experiment 2, by the same operator at each site, following a period of joint training and assessments to minimise differences between BCS assessment between UoN and HAU.

4.4.7: Milk composition, progesterone concentrations and fertility

This study was completed using milk progesterone data that had already been assessed at UoN and HAU composite milk samples (20 ml) were collected over a 12 h period from the pm and following day pm milking at 14 d intervals and analysed as described in Experiment 1 prior to being analysed by NIR for milk fat, protein, SCC and urea concentration at National Milk Laboratories (Wolverhampton, UK). Milk progesterone concentrations were determined as previously described in Experiment 1. The P4 detection limit was 1.5 ng/ml and the mean intra and inter assay coefficients of variation were 7.1 and 8.6%, respectively. The incidence of atypical ovarian activity was determined using milk progesterone concentrations in accordance with Lamming and Darwash (1998) using milk progesterone concentrations

described in Experiment 1 to assess the occurrence of delayed ovulations type I and type II (i.e., pro-longed inter-luteal interval) and persistent CL. The PPI to first P_4 rise, PPI to conception, conception, PR, RR, CO2-eq. were calculated, and animal was evaluated as described in Experiments 1 and 2. In this study, used this data that had been collected previously to allocated the cows into groups that had ACL and TCL.

4.4.8: Statistical analysis

Data was collated in Microsoft Office Excel 2016 and used to calculate weekly mean values for MY, milk fat, protein, lactose, SCC and urea, LW, DMI, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR and PPI to conception and CI. The mean weekly data for MY, corrected MY, milk fat, protein, lactose, SCC and urea, BCS, LW, DMI, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR and PPI to conception and CI were assessed using the Anderson-Darling test for normality of distribution using Minitab statistical software (Version 17.2.1, 2016, State College, PA) and found to be normally distributed except for the milk SCC, which was transformed using the $\log^{base10}$, prior to analysis. The effect of previous diet treatment on the number/proportion of cows, previous milk yield, parity, BCS and LW of cows that had ACL and TCL was assessed using the general linear model (GLM) ANOVA command by each variable/ response using animal as a random effect and previous diet and ACL and TCL as fixed effects in the model. The effect of ACL and TCL were assessed mean BCS was assessed using regression analysis, using BCS as categorical data and ACL and TCL as fixed effect and animal as a random effect in the model. The weekly mean milk yield, composition, FCM, ECM, FPCM, total protein and fat, and milk solids (MS) and lactose, feed intake, BCS, feed efficiency, milking frequency, animal activity and calculated CO_2 eq. were analysed using the General Linear Mixed Model (GLM) command in ANOVA by each variable/ response using animal as a random effect and ACL and TCL as fixed effects in the model. The number of cows that had ACL and TCL, conception rate and the number of inseminations were assessed using Chi-square test, using ACL and TCL as row and response as a column in the table. Data were reported as means (± SE) with P values and the existence of significant differences between means and median were reported at a $P<0.05$, while tendencies were reported at $P<0.10$. The relationship between variables was assessed using Person's correlation and the strength of any correlation between ovarian cycle length and the key performance indicators of fertility, body condition score (BCS) and pp change in BCS, milk yield, feed intake, and feed efficiency were assessed, and the strength of these relationships using R values from which little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high ($\geq$ 0.800) correlation relationships are presented in Tables.

4.5: Results

4.5.1: Reproductive performance

Table 4.2: Mean reproductive performance (± se) of dairy cows that had atypical and typical oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Observations, No. cows	26	62	-
PPI to first pp P4 rise, d[1]	54.7 (2.51)	24.4 (1.63)	<0.001
Mean cycle length	39.5 (2.11)	24.5 (2.80)	0.047
PPI to first artificial insemination, d[1]	78.2 (4.18)	66.2 (2.52)	0.016
Conception rate to first insemination, %	17.0 (4.1)	16.3 (5.0)	0.814
PPI to conception, d[1]	95.2 (6.80)	89.0 (3.92)	0.433
Artificial insemination / conception, No.	2.1 (0.17)	2.7 (0.11)	0.002
Pregnancy rate (PR), %	35 (9.7)	44 (6.3)	0.437
Calving interval [2], d	378 (6.80)	372 (3.92)	0.433

[1] PPI - postpartum interval [2] Calving interval was calculated from PPI to conception, plus a 283 d of gestation.

Table 4.3: Pearson correlation R values[1] between ovarian cycle length and key performance indicators of fertility

	Ovarian CL, d	PPI 1st >P4 rise, d	PPI to 1st AI, d	CR, %	PPI to conception, d	No AI/ conception	PR, %
PPI 1st >P_4 rise, d	-0.339						
PPI 1st AI, d	0.562	0.334					
Conception rate (CR), %	-0.352	-0.280	-0.518				
PPI Conception, d	0.145	0.327	0.454	0.446			
AI / conception, No.	-0.404	-0.306	-0.493	1.000	0.446		
Pregnancy rate (PR), %	-0.027	-0.012	0.072	0.000	0.000	0.051	
Calving interval (CI), d	0.145	0.327	0.454	0.446	1.000	0.446	-0.000

[1] - R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high ($\geq$ 0.800) correlation

The occurrence of ACL in this study was 30 % and the cycle length was 15.0 d longer, PPI to first P4 rise was 30.3 d longer, PPI to the first insemination was 12 d longer and the number

of inseminations required per conception was 0.6 greater (Table 4.2) compared with cows that had TCL. The CR, PPI to conception, PR and CI did not differ between cows that has ACL and TCL. The correlation between ovarian cycle length and the key indicators of fertility was very weak, except for PPI to first AI and between the number of artificial inseminations and conception rate (Table 4.4).

4.5.2: Body condition score and live weight

The BCS and LW and pp change in BCS and LW of cows that had ACL did not differ from cows that had TCL, except for an increase in LW that was greater by cows that had TCL between 60 and 120 and 0 and 120 d pp (Table 4.4).

Table 4.4: Range and mean of body condition score and live weight (± se) of dairy cows that had atypical and typical oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Range in body condition (0 to 5) [1]			
At parturition, 0 d pp	2.50 to 3.00	2.50 to 3.00	0.956
At 60 d pp	2.25 to 2.75	2.5 to 3.75	0.453
At 120 d pp	2.50 to 3.25	2.50 to 3.25	0.845
Body condition (0 to 5) [1]			
At parturition, 0 d pp	2.7 (0.08)	2.7 (0.05)	0.936
At 60 d pp	2.6 (0.10)	2.7 (0.06)	0.443
At 120 d pp	2.8 (0.11)	2.8 (0.07)	0.854
Change in body condition (0 to 5)			
0 to 60 d pp	-0.08 (0.064)	-0.01 (0.042)	0.314
60 to 120 d pp	0.16 (0.053)	0.10 (0.035)	0.320
0 to 120 d pp	0.08 (0.070)	0.09 (0.046)	0.868
Live weight, kg			
At parturition, 0 d pp	628 (13.3)	623 (8.6)	0.348
At 60 d pp	635 (12.0)	625 (7.9)	0.509
At 120 d pp	640 (11.9)	643 (7.8)	0.845
Liveweight change, kg			
0 to 60 d pp	-2.7 (5.81)	2.8 (3.79)	0.427
60 to 120 d pp	5.0 (4.36)	17.3 (2.85)	0.020
0 to 120 d pp	2.2 (6.87)	20.1 (4.49)	0.032

[1] – Body condition score assessed on a 0 to 5 points scale, with 0.25-point intervals (AHBD dairy 2020)

The correlation between ovarian cycle length and BCS and pp change in BCS was very weak (Table 4.5). There were moderately high positive correlations between BCS at parturition (0 d pp) and 60 d pp and 130 d pp, and between 60 d pp and 130 d pp. The correlation between ovarian cycle length and LW, and pp change in LW was very weak (Table 4.6), however there as a high positive correlation between LW at parturition (0 d pp) and 60 d pp, and a moderately high correlation between the change in LW between 0 to 60 d pp and 0 to 130 d pp.

Table 4.5: Pearson correlation R values[1] between ovarian cycle length and body condition score and postpartum change in body condition score

	Ovarian cycle	Body condition			Body condition change	
	length, d	0 d pp	60 d pp	130 d pp	0 to 60 d	60 to 130 d
Body condition						
0 d pp	-0.026					
60 d pp	-0.068	0.746				
130 d pp	-0.002	0.779	0.876			
Change in body condition						
0 to 60 d pp	-0.071	-0.118	0.573	0.348		
60 to 130 d pp	0.114	0.276	0.023	0.502	-0.306	
0 to 130 d pp	0.026	0.102	0.544	0.704	0.686	0.483

[1] - R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high (≥ 0.800) correlation

Table 4.6: Pearson correlation R values[1] between ovarian cycle length and live weight and postpartum liveweight change

	Ovarian cycle	Live weight, kg			Liveweight change, kg	
	Length, d	0 d pp	60 d pp	130 d pp	0 to 60 d pp	60 to 130 d pp
Live weight, kg						
0 d pp	-0.052					
60 d pp	-0.075	0.908				
130 d pp	-0.056	0.481	0.529			
Liveweight change,	kg					
0 to 60 d pp	-0.007	-0.436	-0.035	-0.043		
60 to 130 d pp	-0.023	-0.167	-0.193	0.120	-0.084	
0 to 130 d pp	-0.021	-0.466	-0.151	0.041	0.772	0.568

[1] - R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high (≥ 0.800) correlation

4.5.3: Milk yield and composition, feed efficiency and carbon equivalent contribution

The MY, FC, ECM, FPCM, milk fat concentrations, milk fat, protein, milk solids, log^{base10} SCC, and parity of cows did not differ between cows that had ACL and TCLs (Table 4.7). The mean feed intake and the efficiency with which feed was converted into ECM, FPCM and milk protein and amount of ECM, FPCM and milk protein that was produced per kg DMI did not differ between cows that had ACL and TCL (Table 4.8). However, the DMI during the first 14 d pp was 1.12 kg (± 0.67) lower than cows that had TCL.

Table 4.7: Mean yield and composition (± se) of milk from dairy cows that had atypical and typical oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Milk yield, kg/d	42.2 (1.29)	41.2 (0.84)	0.532
FC milk yield, kg/d [1]	43.4 (1.54)	44.3 (1.00)	0.643
ECM yield, kg/d [2]	45.9 (1.35)	46.1 (0.90)	0.909
FPCM yield, kg/d [3]	42.4 (1.24)	42.6 (0.81)	0.874
Milk fat, g/kg	41.5 (1.20)	43.3 (0.80)	0.222
Milk protein, g/kg	31.9 (0.53)	32.8 (0.44)	0.163
Milk fat, kg/d	1.74 (0.061)	1.77 (0.040)	0.634
Milk protein, kg/d	1.34 (0.039)	1.35 (0.024)	0.862
Milk solids, kg/d	3.07 (0.093)	3.12 (0.060)	0.701
Log^{base10} SCC, cells/ml	1.56 (0.041)	1.50 (0.030)	0.362
Parity, No.	2.19 (0.241)	2.65 (0.156)	0.119

[1] – FC – Fat corrected milk yield, kg = Milk yield kg x milk fat kg / 40.0 [2] – EC – Energy corrected yield, kg = 0.327 x milk yield kg + 12.95 x milk fat kg + 7.21 x milk protein kg (Sjaunja et al. 1990) [3] – FPCM = Milk kg × ((0.1226 × Fat %) + (0.0776 × Protein %) + 0.2534) (Gerber et al. 2010)

The correlation between ovarian cycle length (OCL) and ECM, EPCM yield, mean dry matter intake (DMI) and the feed efficiency (FE) with which milk produced from mean DMI was low. There was little to no correlation between ovarian cycle length and milk yield, dry matter intake, and feed efficiency (Table 4.9), however, there was a moderate correlation between FE of milk protein and FE of ECM and EPCM yield / kg DMI.

Table 4.8: Mean feed intake (DMI) (± se) and feed efficiency of dairy cows that had atypical and typical oestrus cycle length

	Cycle length		P value
	Atypical	Typical	
Feed intake, kg DM/d	22.9 (0.56)	23.7 (0.36)	0.195
EC milk yield/kg total DMI, kg[1]	1.13 (0.016)	1.14 (0.010)	0.481
FPCM yield/kg total DMI, kg[2]	1.04 (0.015)	1.05 (0.010)	0.440
Milk protein/kg dry matter intake, g	32.8 (0.61)	33.3 (0.39)	0.491
Total dry matter intake/kg ECM, kg	0.89 (0.013)	0.88 (0.008)	0.519
Total dry matter intake/kg FPCM, kg	0.97 (0.014)	0.96 (0.009)	0.470
Total dry matter intake/kg milk protein, kg	29.7 (0.47)	28.3 (0.37)	0.487

[1] – EC – Energy corrected milk yield, kg = 0.327 x milk yield kg + 12.95 x milk fat kg + 7.21 x milk protein kg (Sjaunja et al. 1990) [2] – Fat and protein corrected milk yield *(*FPCM) = Milk kg × ((0.1226 × Fat %) + (0.0776 × Protein %) + 0.2534) (Gerber et al. 2010)

Table 4.9: Person correlation R values[1] between ovarian cycle length and milk yield, dry matter intake (DMI) feed efficiency

	Ovarian cycle length, d	ECM yield, kg	EPCM yield, kg	DMI, kg/d	ECM yield/DMI	EPCM yield/DMI
ECM milk yield, kg/d	0.003					
EPCM milk yield, kg/d	0.005	1.000				
DMI, kg/d	0.069	0.501	0.503			
ECM yield/DMI, kg/kg	0.057	0.557	0.571	0.348		
EPCM yield/DMI, kg/kg	0.059	0.541	0.556	0.341	0.999	
Milk protein/DMI, g/kg	0.061	0.160	0.173	0.250	0.602	0.611

[1] - R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high (≥ 0.800) correlation

The replacement rate, additional days over a 365 d CI and related kg of CO_2-eq. related to the additional days CI, replacement rate, and infertility did not differ between cows that had ACLs and TCLs (Table 4.8). The correlation between ovarian cycle length and RR, days over a 365 d CI and CO_2-eq. related to RR, CI and infertility (Table 4.9), however the CO_2-eq. related to infertility was very highly correlated with CO_2-eq. related to RR.

Table 4.8: Mean replacement rate, additional days of calving interval and related carbon equivalent (± se) of dairy cows that had atypical and typical oestrus cycle length

	Cycle length		
	Atypical	Typical	P value
Replacement rate, %	65 (9.7)	56 (6.3)	0.443
Days over 365 d calving interval (CI), d	13.2 (6.80)	7.0 (3.92)	0.442
CO2-eq. for additional days CI, kg [1]	197 (101.0)	104 (58.5)	0.442
CO2-eq. replacement rate, kg [2]	3184 (473.1)	2749 (307.0)	0.443
CO2-eq. infertility, kg [3]	3252 (462.1)	2795 (299.0)	0.408

[1] – Calculated using an assumed replacement rate of 20% and CI of 365 d to calculate additional CO2 eq. /d of addition CI using CO2-eq of 14.9 kg/d CI and addition replacements at CO2-eq. 48.7 kg / % increase in replacement rate [2] – Replacement rate calculated assuming a replacement rate of 20% and CI 365 d using CO2 eq. of 48.7 kg / % increase in replacement rate [3] – Infertility was calculated as the combined [1] addition days of calving interval and [2] replacement rate

Table 4.9: Person correlation R values[1] between cycle length and replacement rate (RR), days over a 365-d calving interval (CI) and CO_2-eq. related to CI, RR and infertility

	Cycle length	RR %	Days over 365 d CI	CO_2-eq. RR, %	CO_2-eq. infertility
Replacement rate (RR), %	-0.027				
Addition days calving interval (CI), d	0.145	*			
CO_2-eq. additional days CI, kg [2]	0.145	*	1.000		
CO_2-eq. replacement rate (RR), kg [3]	0.027	-1.000	-0.000		
CO_2-eq. infertility, kg [4]	0.036	-0.997	1.000	0.997	-0.119

[1] – R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high ($\geq$ 0.800) correlation [2] – Calculated using a RR of 20% and CI of 365 d to calculate additional CO2 eq. /d of addition CI using CO2-eq of 14.9 kg/d CI and addition replacements at CO2-eq. 48.7 kg / % increase in replacement rate [3] – RR calculated assuming a n RR of 20% and CI 365 d using CO2 eq. of 48.7 kg / % increase in replacement rate [4] – Infertility calculated as combined [2] and [3]

4.6: Discussion

4.6.1: Reproductive performance

The occurrence of ACL was relatively common, increasing the PPI to first P4 rise, ovarian cycle length, PPI to the first insemination and the number of inseminations required per

conception. Both delayed cyclicity and the cessation of cyclicity had statistically significant correlations with calving season, calving interval group, and type of P4 profile in previous lactation (Petersson et al. 2006). Calving to first heat was delayed in cows with delayed cyclicity by 12 days (P 0.01) compared to cows with normal P4 profiles, and calving to first service was delayed in cows with any abnormal P4 profile (P 0.01) (Nyman et al. 2014). In this study the CR, PPI to conception, PR, and CI did not differ between cows that had ACL compared with TCL, however the PPI to conception was greater than 80 d. Cows with longer days to first heat and days to first service are expected to also have longer days open because days to first heat, days open, and days to first service are associated traits, high genetic correlations were found between days to first heat, days open, and days to first service therefore, these seem to be various measures of what is in fact the same trait (Pryce et al. 1999). The PR was low at 130 d pp. Compared to cows with normal postpartum ovarian cycles, cows with longer luteal phases had lowered rates of AI submission, conception within 100 days postpartum, and pregnancy within 100 days postpartum (Shrestha et al. 2005). The correlation between ovarian cycle length and the key indicators of fertility was low, except for PPI to first AI and between the number of artificial inseminations and conception rate. All indices of fertility were found to have a positive phenotypic correlation with the interval PP ovulation (Lamming and Darwash, 1998). The genetic correlation between the abnormal reproductive patterns and calving interval and interval between calving and first insemination ranged between 0.27 (±0.07) and 0.54 (±0.05), for the trait total the genetic correlation with calving interval was 0.74 (±0.10), and the genetic correlation between the trait total and PPI first insemination was 0.90 (±0.08) (Van Binsbergen et al. 2019).

4.6.2: Body condition score and live weight

The BCS and pp change in BCS cows that had ACL did not differ from cows that had TCL and was well within industry recommended pp BCS. According to Dillon et al. (2006) cows that have a low BCS at calving and/or those that experience excessive pp BCS loss during early lactating are less likely to ovulate, have lower artificial insemination submission rates, CR to first-service and 6-week in-calf rates and are more likely to experience pregnancy loss and have longer PPI to conception. Cows with delayed cyclicity exhibited extended periods of negative energy balance, lower live weight, and the greatest pp BCS losses (Taylor et al. 2003). Other studies that found no difference in the BCS at parturition and at 21 d pp, however found that cows that had a delayed first ovulation had a considerably lower BCS much later at 5, 7, 9, and 11 weeks pp and that this was related to lengthy luteal phases (Shrestha et al. 2005). Conversely, cows with a BCS of 3.0 had a higher chance of experiencing dystocia or a retained placenta (Hoedemaker et al. 2009), however, cows in this study had a BCS at parturition well within industry recommended standards (AHDB, 2020). Equally, the pp

changes in BCS following parturition were minimal, changes in BCS prior to and after parturition Shrestha et al. (2005) found that cows that had greater BCS losses at 21 d pp had a greater risk of delays in the resumption of ovarian cyclicity and delayed first ovulation. However, in this study the pp changes in BCS and correlation between ovarian cycle length and BCS and pp change in BCS was low. In this study there were moderately high positive correlations between BCS at parturition (0 d pp) and 60 d pp and 130 d pp, and between 60 d pp and 130 d pp. Other studies have noted a significant correlation between BW and BCS, days pp and parity (Meikle et al. 2004), however in this study parity had no effect.

4.6.3: Milk yield and composition,

The MY, FC, ECM, FPCM, milk fat concentrations, milk fat, protein, milk solids, $\log^{base10}$ SCC, and parity of cows did not differ between cows that had ACL and TCLs and there was little to no correlation between ovarian cycle length and ECM and FPCM yield. Shrestha et al. (2005) equally found no effect of the 305 d MY on the resumption of ovarian activity, prolonged luteal phase and delayed first ovulation. While in terms of genetic correlations, Veerkamp et al. (2000) found PPI to luteal activity was highly negatively associated with milk, fat, and protein yields (0.51, 0.65, and 0.48, respectively). Moreover, Bell and Wilson (2018) found that lower milk solids were associated with an increase in survival and a decrease in CO_2-eq emissions per cow and per unit

4.6.4: Dry matter intake and feed efficiency

The feed intake and efficiency with which feed was converted into ECM, FPCM and milk protein and the amount of ECM, FPCM and milk protein that was produced per kg DMI did not differ between cows that had ACL and TCL. The pp NEB has been found to be correlated with the interval from calving to first ovulation (Beam and Butler, 1999) of which DMI is an important element and cows that ovulate later for the first time have been found to have lower DMI compared with those that ovulated earlier during the postpartum period and equally produced more FCM yield (Lucy et al. 1992). While the cows in this study that had similar mean DMI, the cows that had ACL had lower DMI during the first 14 d pp. Lucy et al. (1992) also reported that, compared to multiparous cows, primiparous cows had lower NEB and tended to have longer intervals until first ovulation, but the average time to pregnancy was the same for both types of cows. There was little to no correlation between ovarian cycle length and dry matter intake, and feed efficiency, however, there was a moderate correlation between FE of milk protein and FE of ECM and EPCM yield / kg DMI.

4.6.5: Carbon equivalent contribution of fertility

The PPI to conception and calculated CI was greater than 365 d and the PR was low at 130 d pp, which resulted in a high RR in cows that had ACL and TCL. The CO_2-eq. related to the additional days CI and replacement rate was substantial. The CO_2-eq. associated with RR, CI related infertility, was very highly correlated with CO_2-eq. related to RR. Methane emissions under milk restrictions fall off exponentially as milk yield rises, methane emissions inside the milk yield levels fall with increased fertility (Garnsworthy, 2004).

4.7: Conclusions

In dairy cows, ACL is relatively common occurring in a similar proportion of cows at UoN and HAU, where it delayed the PPI to first milk P4 rise and first insemination and increasing the number of inseminations required per conception. This study showed that a moderate correlation existed between ovarian cycle length and the PPI to the first AI and between the CR and the number of inseminations required per conception. These have the potential to substantially increase the cost of breeding and the risk of untimeliness of calving, which can substantially increase the CO_2-eq and financial cost of milk production. The BCS of cows at parturition, and BCS and LW, and pp change in BCS and LW of cows continued not to be within industry guidelines and not to affect, be related to ACL or be correlated with cycle length. In this study the PPI to conception, calving interval (**CI**), MY, ECM, and FPCM yields, BCS, LW, DMI, FE, RR, and CO_2-eq. related to RR, CI and infertility did not differ between cows that had ACL and TCL. However, cows (ACL and TCL) produced substantial amounts of CO_2-eq. that was related to additional days of CI and RR, which were mainly due to and highly correlated with RR in this study.

4.8: Future work

The occurrence of ACL was relatively common in high-yielding dairy cows and has a negative effect on PPI to first milk (P4) rise and first insemination, number of inseminations required per conception and CR at 130 d pp, which have substantial implications for CO_2-eq related to RR. However, in this experiment MY and FE were not greater in cows that had ACL compared with TCL, which contrasted with Experiment 2. The MY in this study was lower than that in Experiment 2 due to a tendance for MY to continually increase over time. The relationship between MY, DMI and FE, and CO_2-eq. related costs and potential metabolic issues related to diet and feed intake warrant closer examination of a larger group of high-yielding cows in a single location that was completed at a later date than this experiment, to try to assess higher MY and lower the potential variation between sites to assess the effect of MY and FE, on ACL related infertility and CO_2-eq. related to CI and RR.

Appendix 4: Chi square tables of pregnancy rate

Rows: Chi square Rows cycles, Columns: pregnancy rate

	0	1	All
ACL	17	9	26
	15.36	10.64	
TCL	35	27	62
	36.64	25.36	
All	52	36	88

Cell Contents: *Count* *Expected count*

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.605	1	0.437
Likelihood Ratio	0.612	1	0.434

Chapter 5.0

Experiment 4

Effect of atypical ovarian cyclicity on plasma metabolites, feed efficiency, replacement rates, and carbon equivalent of milk produced by high-yielding dairy cows

Effect of atypical ovarian cyclicity on plasma metabolites, feed efficiency, replacement rates, and carbon equivalent of milk produced by high-yielding dairy cows

5.1: Abstract

Increasing the milk yield of dairy cows has been related to less frequent CI, lower PR and greater herd RR. These are major concerns for the sustainability of the global dairy industry, due to the substantial impact these have on the financial cost and CO_2-eq. of milk production. This study aimed to evaluate the ovarian activity, specifically cycle length and the combined effects of CI, RR, MY, FE, and plasma metabolites in relation to fertility and CO_2-eq. related to CI and RR using 97 high yielding dairy cows that had milk progesterone concentrations used to place cows into those that had atypical ovarian cycle length (ACL) and typical cycle lengths (TCLs) to measure the effect on CI, PR, and infertility-rated herd replacement rate (RR) and its effect on MY, FE and CO_2-eq. of milk production and its relationship with plasma metabolites. Cows that had ACL (28.3%) had a longer PPI to first progesterone (P<0.001), cycle length (P<0.001) and tended to have a longer PPI to conception and (P=0.053), while PR and RR did not differ between ACL and TCL at 130 d pp. Cows that exhibited ACLs tended to have greater MY and milk fat concentrations, but FCM, FPCM and milk protein yields and FE did not differ from cows that had TCL. Plasma NEFA, BHB, insulin, glucose and haptoglobin, body condition score at parturition and pp change in BCS did not differ between cows that had ACLs and TCLs and were within industry recommendations. Cows with ACL had more addition days over a CI of 365 d (P=0.046), related RR (P=0.046) and CO2 eq. related to an extended CI compared with TCL, while RR contributed substantially to the CO2 eq. It was concluded that a substantial proportion of cows exhibited ACL, which cows tended to have greater MY and was not affected by BCS and plasma metabolites and had no effect on ECM, FPCM, milk protein yields and FE. The CO2 eq. of milk production was increased by an extended CI and was associated with higher herd RR.

Keywords: Dairy, fertility, Metabolites, Herd replacements, CO2 eq. emissions

5.2: Introduction

Greater individual milk yield (**MY**) of dairy cows has been successful in lowering the enteric greenhouse gas **(GHG)** emissions, by increasing feed efficiency (**FE**) (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014), however, increasing the genetic merit of dairy cows for MY has a deleterious effect on the regularity of ovarian cyclicity (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and pregnancy rates (**PR**) (Brito et al. 2021). These extend the PPI to conception, which increases GHG emissions related to greater calving interval (**CI**) (Bell and Wilson, 2018), while

lower PR (Brito et al. 2021) shortens the length of the productive life of dairy cows (Knaus, 2009) and increases the herd replacement rate (**RR**) and the number of replacements needed (Garnsworthy, 2004; Zehetmeier et al. 2014; Bell et al. 2015), which increases the land, fertilizer, feed, and CO_2-eq. related to the maintenance of non-lactating animals.

The modelling of agricultural systems has shown that the maintenance of greater numbers of herd replacements has been shown to have a substantial negative effect on the carbon footprint of dairy production systems (Garnsworthy, 2004; Bell and Wilson, 2018) along with the results of Chapters 2, 3, Experiment 1 and 2. Greater herd culling and replacement rates substantially affect the financial cost of milk production (Yusuf et al. 2011; Horn et al. 2012; De Vries, 2017; Bell et al. 2015) for farmers. Experiment 2 showed that MY was greater in cows that had ACL. However, there are few published studies that have collected biological data to assess the occurrence of atypical ovarian cycle length (ACL) and the effect on the fertility of high-yielding dairy cows, which include the individual animal evaluation of a wider range of important biological factors such as MY, BCS, feed intake, FE and related plasma metabolites, and subsequent CO_2 eq. of milk production.

5.3: Aim and objectives

This study aimed to assess the effect of atypical cycle length (ACL) in high-yielding dairy cows on fertility-related key performance indicators (KPIs) and CO_2-eq. of milk production and related metabolic indicators. The objectives of the study were to assess the occurrence of ACLs in high-yielding cows and measure the effect of ACL on the CI, PR, and related RR, and to assess the effect MY and FE of high yielding cows on plasma metabolic indicators related to the occurrence of ACL and the potential CO_2-eq.

5.4: Materials and methods

5.4.1: Study location and ethical approval

There were two studies that had been carried out previously between October 2016 and June 2017 and, October 2018 and May 2019 at the UoN Centre of Dairy Science and Innovation (CDSI), Sutton Bonington, Leicestershire, UK as described in Experiment 2.

5.4.2: Experimental design and animal management

The studies were randomised block designs that use 97 Holstein-Friesian dairy cattle of a mean parity of 2.1 (± 0.39; range 1 to 7), live weight (**LW**) of 738.2 (± 25.13) kg and BCS of 3.1 (± 0.22; range 2.50 to 3.50) at parturition that were selected randomly at parturition between October 2016 to May 2017 (48 cows) and October 2018 to April 2019 (49 cows) (Fig

5.1) from 250 cows that calves all year round. Similar proportions of cows from each experiment and from each of the diet treatments (16/17: P=0.892 and 18/19: P=0.932) previously applied had ACL and TCL and the data was used to assess the effect of ACL and TCL (Fig 5.1). In this study the cows were allocated retrospectively according to pp luteal cycle length into blocks that had ACL and TCL lengths as detailed in Experiment 1. The two subsets of cows were housed pens that had 55 free stalls that were fitted with cantilever divisions and in 2016 to 2017 with rubber mats covered with sawdust and 2018 to 2019 with deep litter sand beds, which were cleaned daily, and fresh sand was replenished weekly. The feeding alleys were fitted with solid concrete in 2016/17 and slats between 2018/19, which were cleaned at 6 h intervals using an automatic above-ground scrapper between 2016/17 and robot (Lely UK Ltd., St Neots, UK) and under-floor scrapers between 2018/19 that ran on a 4 h cycle over each 24 h period.

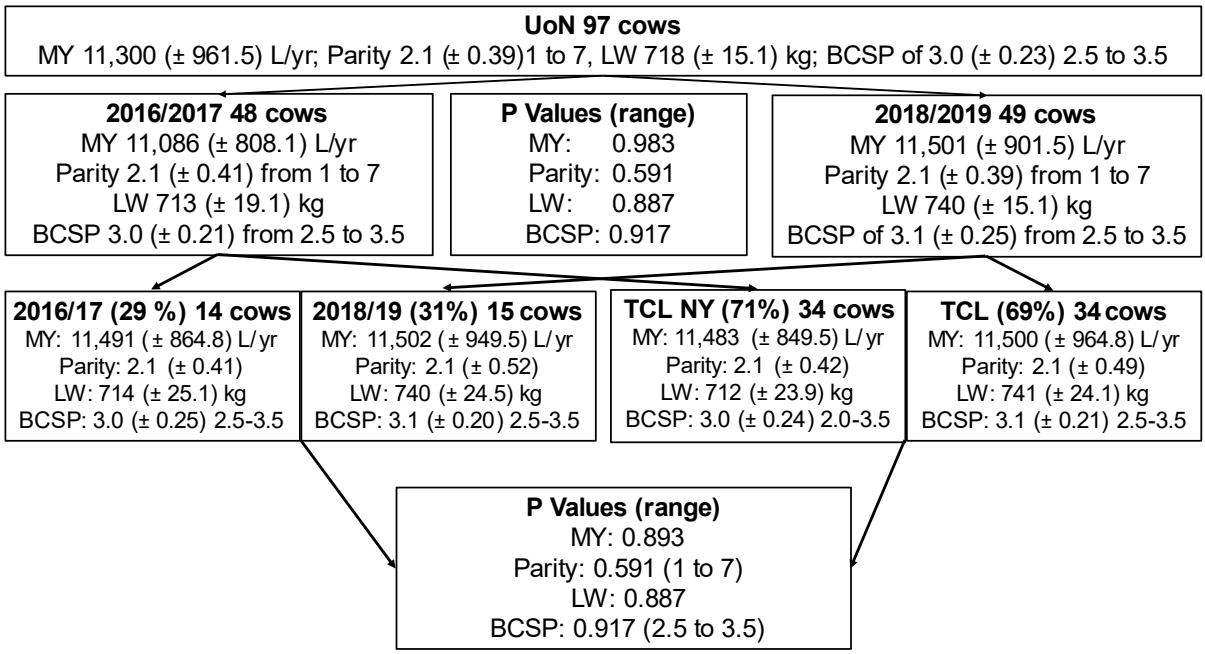


Fig 5.1: Schematic of the proportion of cows that had atypical (ACL) and typical (TCL) cycle lengths from differing experiments completed in 2016/17 and 2018/19 at the University of Nottingham (UoN)

5.4.3: Diets and animal feeding

The cows were offered ad-libitum access to clean fresh water and a partial mixed ration (**PMR**) that was formulated to supply adequate nutrients for the maintenance requirements (**M**), plus 32 L (2016 to 2017) and 30 L (2018 to 2019) of milk production per day (Table 5.1), which was offered as a daily feed allowance of 110% of PMR intake. The PMR was replenished once daily at approx. 07.30 hrs in electronic feed intake stations that were fitted with a feed weighing system (Hokofarm Group B.V., Marknesse, Netherlands), which measured individual animal

feed intake on a constant basis. During milking, cows were offered a pelleted dairy compound feed through the robotic milking station in an integral feed bin (Lely Astronaut A3; Lely UK Ltd., St Neots, UK) and this was distributed at a rate of 2 kg/hd/d at parturition, which increased at 0.2 kg/d up to a maximum of 12 kg /hd/d up to 60 d pp, followed by which allocation remained the same until 100 d pp and then was reduced according to individual animal MY and was offered at 0.4 kg/L on a daily basis.

Table 5.1: Partial mixed ration ingredients and composition of mixed ration and pelleted concentrate offered at milking

	2016 to 2017		2018 to 2019	
	PMR	Concentrates	PMR	Concentrates
Diet components				
Maize silage, kg DM	6.12	-	7.48	-
Grass silage, kg DM	4.01	-	4.89	-
Whole crop silage, kg DM	2.51	-	2.75	-
Wheat straw, kg DM	0.50	-	0.50	-
Soya: Rape seed meal, kg DM	4.70	-	3.56	-
Molasses, kg DM	1.62	-	1.34	-
Protected fat, kg DM	0.45	-	0.36	-
Minerals, kg DM [1]	0.22	-	0.22	-
Diet composition, DM				
Dry matter, %	48.2	87.5	43.8	89.0
Crude protein, %	17.1	18.2	16.8	18.0
Metabolizable energy, MJ/Kg	11.8	12.5	11.7	12.5
NDF, %	35.0	5.1	35.2	5.0
Starch and sugar, %	3.25	35.1	26.1	35.1
Oil, %	4.9	5.0	5.0	5.0
Ash, %	6.7	5.4	7.4	5.3
Diet CO_2-eq. / kg, kg	0.36	1.33	0.35	1.32

[1]-Dietary minerals and vitamins; calcium, 18%; phosphorus, 10%; magnesium, 5%; salt, 17%; copper, 2,000 mg/kg; manganese, 5,000 mg/kg; cobalt, 100 mg/kg; zinc, 6,000 mg/kg; iodine, 500 mg/kg; selenium, 25 mg/kg; vitamin A, 400,000 IU/kg; vitamin D3, 80,000 IU/kg; and vitamin E, 1,000 mg/kg.

5.5: Measurements

5.5.1: Feed samples and feed analysis

As described in Experiment 2 (Table 5.1).

5.5.2: Dry matter intake

Individual animal fresh matter (**FM**) PMR intake was measured using automated electronic feed gates and bins (Hokofarm Group B.V., Marknesse, Netherlands) to record the daily PMR intake, which was used to calculate a weekly mean FM intake and the DM contents (Table 3.1) were used to calculate the individual weekly DMI. The pelleted compound (**PC**) feed that was distributed to each individual cows was recorded by the Lely milking robot (Lely Astronaut A3; Lely UK Ltd., St Neots, UK) and used along with the DM contents (Table 5.1) of the PC to calculate the weekly mean kg of PC DM intake/d. The daily mean PMR and PC DM intake were used to calculate the total daily DMI for each animal.

5.5.3: Milk progesterone

As described in Experiment 2.

5.5.4: Milk yield, live weight and body condition score

As described in Experiment 2.

5.5.5: Plasma metabolites

Blood samples were collected from 18 and 24 of the ACL and TCL cows respectively by suitably experienced and licenced members of the technical support team in accordance with licenced procedures using two x 10 ml vacutainers containing EDTA and sodium fluoride at 09.00 h via jugular venipuncture from individual cow at weekly intervals and placed on ice and following centrifugation plasma stored at -20°C until metabolite analyses. Plasma metabolite concentrations were determined using an Imola Autoanlyser (RX imola; Randox Laboratories Ltd, Antrim, UK) by NUVETNA (Nottingham, UK) using kits (Randox Laboratories Ltd., UK) for the determination of glucose (catalogue no. AS7938), non-esterified fatty acids (NEFA) (catalogue no. FA115), beta-hydroxybutyrate (BOHB) (catalogue no. RB1007), total protein (catalogue no. TP7970), albumin (catalogue no. AB362) and urea (catalogue no. UR3873). While plasma insulin was determined using a non-competitive bovine ELISA kit (Mercodia AB, Uppsala, Sweden) by NUVETNA (Nottingham, UK) that had a detection limit of 0.64 µIU/ml according to the methodology described in ISO11843, Part 4), and the mean intra- and inter-assay coefficients of variation for the QC (10.0 µIU/ml insulin) were 7.6 % and 6.5 % respectively.

5.5.6: Animal health, carbon equivalent impact of infertility

As described in Experiment 2.

5.5.7: Statistical analysis

Data was collated in Microsoft Office Excel 2016 and used to calculate weekly mean values for MY, milk fat, protein, lactose, SCC and urea, LW, DMI, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR and PPI to conception and CI. The mean weekly data for MY, corrected MY, milk fat, protein, lactose, SCC and urea, BCS, LW, DMI, PPI to first P4 rise, PPI to first ovulation, oestrus cycle length between subsequent cycles, number of inseminations, CR, PR and PPI to conception and CI were assessed using the Anderson-Darling test for normality of distribution using Minitab statistical software (Version 17.2.1, 2016, State College, PA) and found to be normally distributed except for the milk SCC, which was transformed using the $\log^{base10}$, prior to analysis. The effect of previous diet treatment on the number/proportion of cows, previous milk yield, parity, BCS and LW of cows that had ACL and TCL was assessed using the general linear model (GLM) ANOVA command by each variable/ response using animal as a random effect and previous diet and ACL and TCL as fixed effects in the model. The effect of ACL and TCL were assessed mean BCS was assessed using regression analysis, using BCS as categorical data and ACL and TCL as fixed effect and animal as a random effect in the model. The weekly mean milk yield, composition, FCM, ECM, FPCM, total protein and fat, and milk solids (MS) and lactose, feed intake, BCS, feed efficiency, milking frequency, animal activity and calculated CO_2 eq. were analysed using the General Linear Mixed Model (GLM) command in ANOVA by each variable/ response using animal as a random effect and ACL and TCL as fixed effects in the model. The number of cows that had ACL and TCL, conception rate and the number of inseminations were assessed using Chi-square test, using ACL and TCL as row and response as a column in the table. Data were reported as means (± SE) with P values and the existence of significant differences between means and median were reported at a $P<0.05$, while tendencies were reported at $P<0.10$. The relationship between variables was assessed using Person's correlation and the strength of any correlation between ovarian cycle length and fertility, body condition score (BCS) and pp change in BCS, milk yield, feed intake, and feed efficiency were assessed, and the strength of these relationships using R values from which little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high ($\geq$ 0.800) correlation relationships are presented in Tables.

5.6: Results

5.6.1: Reproductive performance

Table 5.2: Mean reproduction performance (± se) of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
Observations, No. cows	26	66	-
PPI to first pp P4 rise, d	38.7 (2.45)	23.6 (1.54)	<0.001
Mean cycle length	49.8 (4.21)	21.3 (2.71)	<0.001
PPI to first AI, d	68.6 (4.28)	60.18 (2.76)	0.102
Conception rate, %	80.1 (0.08)	75.8 (0.05)	0.610
PPI to conception, d	83.0 (4.36)	78.7 (5.92)	0.053
Inseminations / conception, No.	2.3 (0.42)	2.1 (0.20)	0.657
Pregnancy rate (PR), %	79 (6.0)	83 (4.3)	0.602
Calving interval (CI)[1], d	397 (7.37)	370 (11.4)	0.053

[1] – Calving interval (CI) – Calculated from PPI to conception + 283 d gestation. [2] – Replacement rate calculated on an assumed replacement rate of 20% and CI 365 d. [3] – Combined replacement rate for addition days of calving interval + 100 % for replacements.

Table 5.3: Person's correlation R values[1] between postpartum ovarian cycle length and mean reproduction performance

	Cycle	Postpartum interval to			Calving
	Length, d	First P4 rise, d	First AI, d	Conception, d	interval, d
PPI first >P4, d	-0.332				
PPI to first AI, d1	0.437	0.439			
PPI to conception, d	0.479	0.196	0.471		
Calving interval, d	0.479	-0.064	0.308	0.810	
Pregnancy rate, %	-0.072	0.042	-0.087	-0.000	-0.000

[1] – R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high ($\geq$ 0.800) correlation

This study found ACL occurred in 28.3 % of the dairy cows and ACLs were 28.5 d longer and the PPI to first P4 rise was 15.1 d longer compared with cows that had TCL (Table 5.2). While there was no difference in the PPI to first AI, CR, PR, PPI to conception and number of AI per

conception by 130 d pp and CI of cows that had ACL than cows that had TCL. The correlation between ovarian cycle length and the PPI first >P4, PPI to first AI, PPI to conception, CI and PR was weak (Table 5.2).

5.6.2: Milk yield, composition, and parity

Table 5.4: Mean yield and composition (± se) of milk of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
Milk yield, kg/d	48.5 (1.52)	44.2 (0.99)	0.098
FC milk yield, kg/d [1]	47.3 (1.45)	46.6 (0.92)	0.678
ECM yield, kg/d [2]	48.3 (1.58)	45.2 (0.99)	0.274
FPCM yield, kg/d [3]	47.3 (1.36)	45.6 (0.86)	0.288
Milk fat, g/kg	39.6 (1.04)	41.7 (0.07)	0.088
Milk protein, g/kg	31.9 (0.05)	32.5 (0.03)	0.413
Lactose g/kg	47.9 (0.09)	48.5 (0.06)	0.561
Urea, %	0.27 (0.006)	0.28 (0.004)	0.290
Milk fat, kg/d	1.89 (0.058)	1.86 (0.037)	0.678
Milk protein, kg/d	1.53 (0.047)	1.46 (0.030)	0.185
Milk solids, kg/d	3.42 (0.098)	3.32 (0.062)	0.377
Log^{base10} SCC, cells/ml	1.68 (0.035)	1.63 (0.043)	0.101
Parity, No.	2.92 (0.327)	2.61 (0.205)	0.413

[1] – FC – Fat corrected yield, kg = Milk yield kg x milk fat kg / 40.0. [2] – EC – Energy corrected yield, kg = 0.327 x milk yield kg + 12.95 x milk fat kg + 7.21 x milk protein kg (Sjaunja et al. 1990). [3] – FPCM = Milk kg × ((0.1226 × Fat %) + (0.0776 × Protein %) + 0.2534) (Gerber et al. 2010).

The MY tended to be greater and the milk fat concentrations tended to be lower in milk from cows that had ACL compared with TCL during the first 130 d pp and mean MY was (P 0.035) greater during the first week pp in cows that had ACL. However, there was no difference in milk fat, protein and SCC, lactose and urea concentrations, FC, ECM, FPCM, milk fat, protein and milk solids yields and parity between cows that had ACL compared with cows that had TCL up to 130 d pp (Table 5.4). The correlation between MY, MF, MP yields, FCMY, ECMY, EPCMY and milk fat and protein concentrations were weak (Table 5.5). However, there was a moderate correlation MY and milk fat (MF) concentrations and a strong relationship between MY, and milk fat and protein concentration and MS, FCMY, ECMY and EPCMY.

Table 5.5: Person's correlation R values[1] between postpartum ovarian cycle length and mean milk yield and composition

	Cycle length, d	MY, kg	MF, g/kg	MP, g/kg	Fat, kg/d	Protein, kg/d	MS, kg/d	FCMY, kg	ECMY, kg
MY, kg/d	-0.039								
MF, g/kg	0.088	-0.511							
MP, g/kg	0.076	-0.408	0.474						
Fat, kg/d	0.025	0.712	0.227	-0.097					
Protein, kg/d	0.003	0.892	-0.331	0.040	0.733				
MS, kg/d	0.016	0.851	-0.026	-0.038	0.945	0.916			
FCMY, kg/d	0.025	0.712	0.227	-0.097	1.000	0.733	0.945		
ECMY, kg/d	-0.000	0.917	-0.142	-0.184	0.924	0.915	0.987	0.924	
EPCMY, kg/d	0.002	0.908	-0.123	-0.161	0.929	0.916	0.991	0.929	1.000

[1] – R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high (≥ 0.800) correlation

5.6.3: Dry matter intake and feed efficiency

Table 5.6: Mean dry matter intake (DMI) (± se), rumination and feed efficiency of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		P value
	Atypical	Typical	
Total dry matter intake (DMI), kg/d	24.2 (0.68)	24.9 (0.43)	0.305
EC yield[1] / kg total DMI, kg	2.03 (0.076)	1.90 (0.048)	0.116
FPCM yield [2] / kg total DMI, kg	1.88 (0.070)	1.75 (0.045)	0.116
Milk protein/kg DMI, g	22.3 (5.09)	24.9 (3.22)	0.668
Total DMI / kg ECM, kg	0.50 (0.016)	0.53 (0.010)	0.113
Total DMI / kg FPCM, kg	0.56 (0.017)	0.58 (0.011)	0.116
Total DMI / kg milk protein, kg	22.3 (1.09)	24.9 (3.22)	0.668

[1] – Energy corrected (EC) kg = 0.327 x milk yield kg+12.95 x milk fat kg+7.21 x milk protein kg (Sjaunja et al. 1990); [2] – FPCM = Milk kg×((0.1226 × Fat %)+(0.0776 × Protein %)+0.2534) (Gerber et al. 2010).

The DMI, FCE and FE did not differ between cows that had ACL and TCL (Table 5.6), however DMI tended (P 0.087) to be lower 1.73 (± 0.89) and 1.46 (± 0.74) kg lower during the first 14 d pp in cows that had ACL and TCL respectively compared with 21 d pp and mean DMI.

5.6.4: Body condition score and live weight

Table 5.7: Range and mean of body condition score and live weight (± se) of dairy cows atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
Range in body condition (0 to 5) [1]			
At parturition, 0 d pp	2.50 to 3.50	2.25 to 3.50	0.411
At 60 d pp	2.25 to 3.00	2.00 to 3.50	0.331
At 120 d pp	2.50 to 3.50	2.50 to 3.50	0.452
Mean body condition (0 to 5) [1]			
At parturition, 0 d pp	3.0 (0.08)	3.0 (0.05)	0.397
At 60 d pp	2.9 (0.09)	3.0 (0.06)	0.263
At 120 d pp	3.0 (0.12)	3.1 (0.07)	0.440
Change in body condition (0 to 5)			
0 to 60 d pp	-0.07 (0.098)	-0.07 (0.061)	0.990
60 to 130 d pp	-0.08 (0.084)	-0.10 (0.052)	0.832
0 to 120 d pp	0.08 (0.084)	0.10 (0.053)	0.832
Live weight, kg			
At parturition, 0 d pp	720 (20.4)	721 (12.8)	0.877
At 60 d pp	716 (18.7)	717 (11.8)	0.846
At 120 d pp	727 (19.1)	728 (12.0)	0.792
Liveweight change, kg			
0 to 60 d pp	-3.07 (1.09)	-4.07 (1.06)	0.990
60 to 120 d pp	11.0 (3.79)	11.4 (2.38)	0.754
0 to 120 d pp	16.5 (6.42)	15.9 (4.03)	0.527

[1] – Body condition: Assessed (0 to 5 points scale, with 0.25-point intervals) according to AHDB, 2021

The LW and BCS did not differ at 0, 60, and 120 d pp and pp change in LW and BCS between 0 and 60, 60 and 120, and 0 and 120 d pp between cows that has ACL and TCLs (Table 5.7). The strength of the relationship between cycle length and BCS at 0, 60 and 120 d pp and pp change in BCS between 0 and 60, 60 and 120 and 0 and 120 d pp were very weak (Table 5.8). There was a moderate relationship between BCS at parturition and BCS at 60 and 120

d pp and between change in BCS between 0 and 60 d pp and a strong relationship between BCS at 60 d pp and 120 d pp and 120 d pp and pp change in BCS between 0 and 120 d pp. There was no difference in the mean plasma NEFA, BHB, glucose and insulin, urea and haptoglobin (Tables 5.9, 5.10 and 5.11) concentrations of cows that had ACL and TCL between 14 and 130 d pp.

Table 5.8: Person's correlation R vaules[1] between postpartum ovarian cycle length and mean milk yield and composition

	Cycle	BCS, d pp			pp change in BCS	
	length, d	0	60	120	0 to 60	60 to 120
BCS, 0 d pp	0.050					
BCS, 60 d pp	-0.080	0.639				
BCS, 120 d pp	-0.037	0.615	0.814			
BCS, 0 to 60 d pp	-0.145	-0.180	0.486	0.323		
BCS, 60 to 120 d pp	0.044	0.102	-0.046	0.422	-0.487	
BCS, 0 to 120 d pp	-0.087	-0.097	0.470	0.722	0.613	0.392

[1] – R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high (≥ 0.800) correlation

5.6.5: Plasma metabolites

Table 5.9: Mean beta-hydroxy butyrate (BHB) (± se) concentrations of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
BHB, mmol/L			
7, d pp	0.832 (0.0842)	0.787 (0.0540)	0.625
14, d pp	0.966 (0.1870)	0.987 (0.1200)	0.925
28, d pp	0.891 (0.1150)	0.864 (0.0720)	0.843
42, d pp	0.733 (0.0757)	0.859 (0.0486)	0.168
56, d pp	0.740 (0.0439)	0.779 (0.0282)	0.463
70, d pp	0.735 (0.0510)	0.800 (0.0332)	0.288
84, d pp	0.681 (0.0471)	0.785 (0.0302)	0.070
98, d pp	0.737 (0.0430)	0.816 (0.0276)	0.172
112, d pp	0.821 (0.0449)	0.778 (0.0278)	0.422

Table 5.10: Mean non-esterified free fatty (NEFA) acid, plasma glucose and insulin (± se) concentrations of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
NEFA, µmol/L			
7, d pp	0.721 (0.1050)	0.603 (0.0670)	0.347
14, d pp	0.356 (0.0525)	0.333 (0.0337)	0.709
28, d pp	0.189 (0.0440)	0.227 (0.0282)	0.476
42, d pp	0.128 (0.0442)	0.221 (0.0283)	0.085
56, d pp	0.165 (0.0329)	0.156 (0.0211)	0.625
70, d pp	0.134 (0.0202)	0.126 (0.0130)	0.754
84, d pp	0.098 (0.0213)	0.134 (0.0137)	0.164
98, d pp	0.095 (0.0120)	0.099 (0.0077)	0.814
112, d pp	0.091 (0.0015)	0.110 (0.0090)	0.727
Glucose, mmol/L			
7, d pp	3.18 (0.127)	3.21 (0.082)	0.851
14, d pp	3.26 (0.158)	3.25 (0.101)	0.958
28, d pp	3.34 (0.120)	3.36 (0.077)	0.923
42, d pp	3.41 (0.097)	3.42 (0.062)	0.895
56, d pp	3.44 (0.079)	3.46 (0.051)	0.804
70, d pp	3.46 (0.075)	3.37 (0.048)	0.341
84, d pp	3.37 (0.062)	3.36 (0.040)	0.913
98, d pp	3.28 (0.095)	3.35 (0.061)	0.543
112, d pp	3.35 (0.090)	3.44 (0.055)	0.401
Insulin, mg/L			
7, d pp	0.29 (0.112)	0.42 (0.072)	0.336
14, d pp	0.58 (0.107)	0.60 (0.068)	0.823
28, d pp	0.52 (0.104)	0.69 (0.067)	0.169
56, d pp	0.65 (0.109)	0.67 (0.070)	0.856
98, d pp	0.81 (0.124)	0.75 (0.077)	0.648
112, d pp	0.82 (0.121)	0.78 (0.098)	0.701

Table 5.11: Mean plasma and urea and haptoglobin concentrations (± se) of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
Urea, mg/L			
7, d pp	3.19 (0.192)	3.25 (0.123)	0.797
14, d pp	3.38 (0.189)	3.48 (0.121)	0.171
28, d pp	4.06 (0.201)	3.91 (0.126)	0.530
42, d pp	4.30 (0.193)	3.95 (0.124)	0.126
56, d pp	4.45 (0.176)	4.22 (0.113)	0.285
70, d pp	4.47 (0.195)	4.39 (0.127)	0.738
84, d pp	4.68 (0.195)	4.63 (0.125)	0.818
98, d pp	4.93 (0.236)	4.92 (0.151)	0.980
112, d pp	4.65 (0.225)	4.76 (0.139)	0.700
Haptoglobin, µg/L			
7, d pp	0.39 (0.099)	0.44 (0.064)	0.703
14, d pp	0.24 (0.062)	0.32 (0.040)	0.257
28, d pp	0.18 (0.034)	0.18 (0.021)	0.990
42, d pp	0.17 (0.040)	0.19 (0.026)	0.639
56, d pp	0.14 (0.087)	0.26 (0.056)	0.221
70, d pp	0.19 (0.077)	0.24 (0.050)	0.555
84, d pp	0.12 (0.030)	0.16 (0.019)	0.246
98, d pp	0.15 (0.031)	0.13 (0.020)	0.689
112, d pp	0.19 (0.073)	0.20 (0.045)	0.920

5.6.6: Carbon equivalent related to infertility

The number of days over a 365 CI and RR for additional days over a 365 d CI and CO2-eq. related to infertility were greater in cows that had ACLs compared with TCLs (Table 5.12), while the RR, infertility related to RR and CO_2-eq. related to her replacements and infertility did not differ between cows that had ACLs and TCLs. There was a relatively weak relationship between cycle length and the number of additional days over a 365 d CI, RR and additional RR related to additional days of CI and between cycle length and CO_2-eq. CI, CI RR, RR and infertility (Table 5.13). There was a strong correlation between CO_2-eq. related to RR and CO_2-eq. related to infertility of dairy cows.

Table 5.12: Carbon dioxide equivalent (± se) of dairy cows that had atypical and typical postpartum ovarian cycle length

	Cycle length		
	Atypical	Typical	P value
Days over 365 d calving interval (CI), d	32.0 (11.40)	5.3 (7.37)	0.049
Replacement rate days over 365 d CI, %	1.75 (0.404)	0.29 (0.623)	0.049
Replacement rate, %	24.2 (5.22)	19.2 (8.31)	0.602
Infertility-related replacements, %	25.6 (5.15)	19.5 (8.21)	0.531
Infertility-related CO_2-eq. < 160 d pp			
CO_2-eq. for days over 365 d, kg	476 (110.0)	79 (169.0)	0.049
CO_2-eq. for herd replacements CI, kg	1181 (254.0)	937 (405.0)	0.611
CO_2-eq for infertility, kg	1606 (251.0)	1012 (400.1)	0.212

[1] – Calculated using an assumed replacement rate 20% and CI of 365 d used to calculate additional replacements [2] – Combined CO_2-eq for additional herd replacements and calving interval greater than 365 and 375 days

Table 5.13: Person's correlation R values between postpartum ovarian cycle length and mean milk yield and composition

	Cycle	Additional		Additional	CO_2-eq.		
	length, d	CI, d	RR	RR, CI	CI	CI RR	RR
Additional CI, d	0.479						
Replacement rate (RR), %	0.072	*					
Additional RR, CI	0.479	1.000					
CO_2-eq. CI, d	0.479	1.000	1.000	1.000			
CO_2-eq. CI RR	0.479	1.000	1.000	1.000	1.000		
CO_2-eq. RR	0.072	0.000	1.000	-1.000	-1.000	1.000	
CO_2-eq. infertility	0.151	1.000	0.917	1.000	1.000	1.000	0.917

[1] – R values: Little to weak (0.000 to 0.400); moderate (0.500 to 0.700) and high ($\geq$ 0.800) correlation

5.7: Discussion

5.7.1: Cycle length and fertility

In this study, ACL occurred in 28.3 % of the dairy cows. Cycles were 28.5 d longer and the PPI to first P4 rise was 15.1 d longer compared with cows that had TCL, which was in keeping

with Taylor et al. (2003), who found that the main factor associated with ACL was a delay in P4 rise and ovulation. In this study, PPI to first AI was delayed by 12 d in cows that had ACL compared with TCL, and this delay in first service was equally noted by Nyman et al. (2014) in cows that had a delay in first P4 rise and ACL. Petersson et al. (2006) found that in cows with delayed pp ovarian cyclicity, had a longer PPI to the beginning of luteal activity and that this was reflected in the PPI to first oestrus, which was stated to be considerably longer when cow had a delayed PPI to the onset of ovarian activity. Despite there being no difference in the PPI to first AI, CR and PR of ACL and TCL in this study, these findings indicate the detrimental effect that a delay in the PPI interval to P4 rise and ACL can have on pp fertility and the CI.

5.7.2: Plasma metabolites

There was no difference in the mean plasma NEFA and BHB, glucose and insulin, urea and haptoglobin concentrations of cows that had ACL and TCL between 14 and 120 d pp. Other researchers have found that the plasma glucose concentrations were considerably greater in cows that had persistent CL at the beginning of the service period between 50 and 60 d pp (Taylor et al. 2003). Glucose metabolism, secretions and motility of the gastrointestinal tract are regulated by ghrelin, which is involved in a wide spectrum of biological functions, including GH secretion and regulation of EB (Lorenzi et al. 2009) through the promotion of DMI (Korbonits et al. 2004) and maintenance of metabolic homeostasis (Hidekitamura et al. 2000; Nakazato et al. 2001; Hattori et al. 2001). However, there was no difference in plasma glucose concentrations between cows that had ACL and TCL in this study. The LW and BCS at parturition and 60, and 120 d pp and pp change in LW and BCS between 0 and 60, 60 and 120, and 0 and 120 d pp did not differ between cows that has ACL and TCL. This was supported by plasma concentrations of NEFA and BHB were similar in cows that had ACL and TCL. Taylor et al. (2003) found that cows that ACL had delayed ovulation experienced considerably greater pp LW loss, higher BHB concentrations and tended to have the lower insulin and glucose concentrations compared with those that had TCL. This was not the case in this study, in which cows that had delayed in P4 rise and ACL were cows that had minimal pp LW and BCS change that was similar in cows that had ACL and TCL, as were plasma BHB, NEFA and insulin and glucose concentrations.

5.7.3: Milk yield, feed intake and energy balance

While the MY tended to be greater in cows that had ACL, there was no difference in FC, ECM. PFCM, milk fat, protein and SCC, lactose and urea concentrations, milk fat, protein and milk solids yields or parity of cows that had ACL compared with cows that had TCL up to 130 d pp in this study. These results reflected those of Shrestha et al. (2005) who also found that the

305-day milk yield of cows that had ACL and TCL were similar. This study found that the mean DMI and FE did not differ between cows that had ACL and TCL, however, cows that had ACL had lower DMI over the first and second weeks pp. Feed intake has been indicated as a principal factor affecting fertility (Webb et al. 2004; Robinson et al. 2006; Ashworth et al. 2009) amongst other factors, which include BCS (Roche et al. 2009), MY and milk composition (Friggens et al. 2007), plasma metabolite and hormone concentrations (McDougall et al. 2005). However, this study found no difference DMI, BCS, ECM and PFCM, milk fat, protein and solids yields, FE or plasma metabolites between cows that had ACL and TCL. There have been other studies that had found that NEFA, T-cholesterol, total protein, and urea nitrogen did not differ in cows that has ACL, up until 3 to 5 weeks pp, when plasma NEFA concentrations were greater in cows that had delayed ovulation (Shrestha et al. 2005), however this was not the case in this study, with NEFA concentrations being similar at 28 and 42 d pp between cows that had ACL and TCL.

5.7.4: Carbon dioxide equivalents related to calving interval and herd replacement rate

The number of days over a 365 CI and RR attributed to additional days over a 365 d CI and CO2-eq. related to infertility were greater in cows that had ACLs compared with TCLs, while in this study the RR, infertility related to RR and CO_2-eq. related to herd replacements and infertility did not differ between cows that had ACLs and TCLs. Other studies have modelled UK data to show how improvements in oestrus detection and conception can lower methane emissions related to RR (Garnsworthy, 2004) and CO2-eq. related to milk production can be reduced by achieving a CI closer to 365 d and reducing RR (Bell and Wilson, 2018). The study provides biological data that supports these findings, demonstrating that substantial amounts of CO2-eq. is related to an extended CI and related RR due a delay in the pp P4 rise and ACL.

The data from this study supports these findings, demonstrating that substantial reduction in CO2-eq. related to lowering the CI and RR may be achieved by improvements in pp resumption of ovarian activity. This may be achieved by improving pp DMI during the first 14 d pp by optimization of diet composition in relation to the average requirements of the dairy cows in the feeding group to achieve a higher levels of DMI (Fish et al. 2012; Llonch et al. 2018; Rumphorst et al. 2022). The inclusion of rumen-protected choline and dry propylene glycol in cows diets during the pre and pp transitions period (Chung et al. 2009). Moreover, making changes to animal management much earlier in the life of dairy heifers by applying group rearing of calves, which improves calf feed intake, health and survival (Costa et al. 2015 and 2016) but more importantly increases the resilience of dairy cattle to regrouping, especially around first parturition (Zhang et al. 2022). While the genetic selection of cows for

fertility traits in addition to MY and FE continues to improve the PR and fertility of dairy cows (Pryce et al., 2001; Lucy, 2019; Brito et al., 2021).

5.8: Conclusions

The data from this study supports these findings, demonstrating that substantial reduction in CO2-eq. related to lowering the CI and RR could be achieved by improvements in pp resumption of ovarian activity.

5.9: Further work

The assessment of methane emissions and CO_2-eq. of herd replacements and management factors that may increase the DMI of cows during the first 14 d pp need to be evaluated including group heifer rearing and early lactation, effect of peripartum feeding systems and diets.

Appendix 4: Chi-Square

Table: Chi-Square Rows: Cycle length Columns: Conception / Pregnancy rate

	0	1	All
ACL	5	21	26
	5.93	20.07	
TCL	16	50	66
	15.07	50.93	
All	21	71	92

Cell Contents: Count Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.266	1	0.606
Likelihood Ratio	0.273	1	0.602

Chapter 6

General discussion

6.0: General discussion

6.1: Carbon footprint of dairy production

Dairy production has been cited as a major greenhouse gas (GHG) emitter, which is contributing to climate change (Caro et al. 2014). Life cycle analysis (LCA) has shown, not considering carbon emissions from land use/land use change, that the production of methane (CH_4) from enteric fermentation and feed production, processing, transport, and storage, and the rearing of herd replacements are the main determinants of GHG emissions (Flysjö et al. 2012; Wolf et al. 2017). Increasing the MY of individual cows has been successful in enhancing feed efficiency (FE) and diluting enteric GHG emissions related to the production of milk (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014). It is essential to maintain global warming < 1.5°C up to 2030 and achieve net zero emissions by 2050 (UN, 2022), however greater MY per cow has been associated with a decline in cow fertility (FAO, 2005; Butler, 2003; Royal et al. 2000; Moore and Thatcher, 2006; Bello et al. 2012; Brito et al. 2021) and greater GHG emissions related to extended CI and greater numbers of herd replacements (Bell and Wilson, 2018). This thesis set out to measure milk P4 concentrations to evaluate the pp luteal activity and measure the occurrence of ACL and assess the effect of the cycle length in high-yielding dairy cows to measure the occurrence and effect of delayed pp luteal activity on PPI to conception and CI, PR, and RR. This data was used, along with the CO_2-eq. emissions related to each day of CI above 365 d and per % of RR to assess the effect of ACL on CO_2-eq. emissions related to CI and RR and to evaluate the potential relationship between pp luteal activity, MY, FE, and CO_2-eq. related to fertility, specifically the CI and dairy herd RR.

6.2: Milk yield and assurance of atypical ovarian activity

The average MY of the Holstein Friesian dairy cows used in the experimental work included in this thesis was between 10,500 to 11,500 L/cow per year (Exp 2, 3 and 4), which would have MY of 44 to 50 L in early lactation and be in the top 10% of dairy herds from the UK, which are above the average 8,090 l/cow/year in 2021 (AHDB, 2022). In these cows, the milk P4 concentrations that were used to evaluate the pp luteal activity and the effect of the cycle length in high-yielding dairy cows showed that ACL occurred in between 30 and 44 %, with a mean of 33.1% of dairy cows, which was in keeping with previous studies that found that 40% of the dairy cow population had ACL (Petersson et al. 2006; Windig et al. 2008; Cutullic et al. 2011). The 28.3 to 44% of high-yielding cows that exhibited ACL in the studies presented in this thesis were similar to previous studies, indicating that increasing MY and FE did not increase the occurrence of ACL in Holstein Friesian cows. This is important because Holstein

Friesian cows have been found to show more abnormal cyclicity compared with some other breeds, such as Jersey (Petersson et al. 2006; Cutullic et al. 2011; Piccand et al. 2013) and the Holstein Friesian breed is globally the most dominant dairy breed (Margerison, 2022). The lack of an increase in the proportion of high-yielding cows that exhibit ACL and the lack of correlation between MY and ACL presented in this thesis are most likely due to the inclusion of fertility traits being included in breeding programs of dairy cows for several years (VanRaden et al. 2004; Huang et al. 2007) and accounting for between 13 to 30% of the observed variance in the fertility of dairy cows (Veerkamp et al. 2000; Royal et al. 2002b; Petersson et al. 2007). This is likely to be responsible for the potential ability to increase MY while maintaining pp ovarian activity in the majority, 56 to 72 % of the dairy cows assessed in this thesis. This potentially provides an optimistic outlook for the continued dilution of CO2-eq. by increasing MY of individual dairy cows (VandeHaar and St-Pierre, 2006; Capper et al. 2009; Reynolds et al. 2011; Garnsworthy et al. 2012; Knapp et al. 2014), which is especially relevant for developing countries going forwards (Margerison, 2022). However, the deleterious effect of high genetic merit for MY on cyclicity is complex (Horan et al. 2004; Pollott and Coffey, 2008; Windig et al. 2008) and requires the evaluation of the relationship between BCS, animal health, and nutrition, which are important in the evaluation of the occurrence and effect of ACL.

6.3: Body condition score at parturition and atypical ovarian activity

The cows that had ACL and TCL in the studies in this thesis had BCS at parturition that was between 2.6 and 3.0, which are within industry-recommended standards (AHDB, 2020) for dairy cows, making it unlikely that the occurrence of ACL was related to reproductive abnormalities that are more common in dairy cows that have BCS >3.0 at parturition. Moreover, the pp change in BCS of the cows in the studies in this thesis did not differ between cows that exhibited ACL and TCL, making it unlikely that this was a factor that affected the occurrence of ACL. Equally, the cows in these studies were offered a prepartum transition diet that included low amounts of grass silage, along with maize silage, cereal straw, and soyclor and none of the cows included in the studies had clinical hypocalcaemia, metritis, and endometritis, which have been major health issues that contribute to suboptimal fertility due to the failure to resolve uterine inflammation, lack of interaction between GH and IGF-1 (Walsh et al. 2011) making metabolic disorders and uterine disease unlikely contributors to the delay in pp ovarian activity and CR. Equally, the cows in the studies in this thesis were within the BCS at partition recommendations (AHDB, 2022) and the frequency of occurrence of ACL was not due to cows being over-conditioned at parturition (Cutullic et al. 2012; Bedere et al. 2016). The mean DMI did not differ between cows that exhibited ACL and TCL, however, DMI in the first 14 d pp tended and was lower in cows that exhibited ACL and these cows did and tended

to have higher MY in some studies (Exp 2 and 5). This is unlikely to be due to BCS and or pp loss of BCS because they had similar BCS at parturition and the direct effects of body fat on plasma concentrations of leptin (McGregor et al. 1996) and its potent effect on satiety (Campfield et al. 1995) and feed intake, via the neuroendocrine-axis (Cervero et al. 2006) is unlikely to have affected DMI following parturition in cows that had ACL in this thesis. The direct link between nutrition, metabolic status, and reproduction (Clarke and Henry, 1999) may indicate that the short-term early lactation DMI of cows is an area that could provide an opportunity to reduce the occurrence of ACL and prolonged PPI to first P4 rise shown in all the Experiments in this thesis and ovarian activity and improve CR and PR, which could increase the productive life of cows, lowering the CI and RR.

6.4: Postpartum change in body condition score and atypical ovarian activity

The pp change in BCS between 0 and 60 d pp did not differ between cows that had ACL and TCL. The loss of BCS between 0 and 60 d pp was between 0.01 and 0.02, which was minimal and well within recommended standards (AHDB, 2022) of -0.5 points between parturition and 60 d pp. The pp change in BCS was consistently marginal across the studies presented in this thesis and these were well within industry (-0.5 BCS) recommendations (AHDB, 2020). It has been shown that dairy cows that lose < 0.5 pp BCS between 0 to 60 d pp should return to ovarian activity and first ovulation within 30 d pp (Pryce et al. 2001; Garnsworthy, 2006; Patton et al. 2007), which was not the case for cows that exhibited ACL in this thesis. It is however unlikely that BCS at parturition and/or pp loss of BCS presented in this thesis was related to the pp delay in ovarian activity in cows that exhibited ACL, because there was no difference in BCS at parturition, pp change in BCS was minimal and were well within industry standards (AHDB, 2020) compared with other studies that found an effect of BCS on pp ovarian activity (Diskin et al. 2003; Friggens and Chagunda, 2005; Chagas et al. 2007; Leroy, et al. 2008; Aguilar-Perez et al. 2009). These findings were supported by the NEFA, BHB, and insulin concentrations in Experiment 4, which did not differ between cows that had ACL and TCL and were within reference ranges (Leroy et al. 2010a), unlike studies that found cows that lost more BCS pp (> 0.5) affected the growth and development of ovarian follicles (Lucy, 2000; Leroy et al. 2010a) due to NEB, which was associated with greater plasma NEFA concentrations, directly related poor follicular growth (Vanholderet al. 2006; Walsh et al. 2011; Garcia-Garcia, 2012) due to low plasma IGF-1 (Lucy, 2000), insulin concentrations (Wathes et al. 2007; Lucy, 2008; Wathes, 2012) but this was not the case in Experiment 4 in this thesis.

6.5: Postpartum milk yield, feed intake and prolonged commencement of ovarian activity

A delay in the commencement of ovarian activity in cows that had ACL was largely consistent across all the studies in this thesis. This was in keeping with previous studies that showed that ACL most typically delayed the commencement of luteal activity (Petersson et al. 2006; Windig et al. 2008; Cutullic et al. 2011). The PPI to the first P4 rise was delayed by between 7 and 31 d, which resulted in a mean delay of 15.5 d across the studies in this thesis. The pp loss of BCS between parturition and 60 d pp and during the first few weeks pp was unlikely to influence the commencement of luteal activity and the occurrence of prolonged luteal phase may be related to MY (Royal et al. 2002b; Kafi et al. 2012) due to the biological effect of MY on BCS experimental outcomes may be confounded by these factors. However, feed intake has been indicated as the primary factor eliciting the effect of EB on reproduction (Patton et al. 2007). While the mean DMI of cows did not differ in cows that had ACL and TCL, closer examination of DMI during the first few weeks of lactation showed that cows that had ACL had lower DMI in the first two weeks of lactation. Lower DMI and lesser than adequate nutrient supply to meet the requirements for maintenance and milk production have been shown to affect the fertility of dairy cows, but not MY (Friggens et al. 2010; Walsh et al. 2011).

In this study cows that had ACL, had prolonged PPI to first P4 rise and there was a short-term (acute) undernutrition during the first 14 d pp in cows that had ACL, which has been shown to suppress gonadotrophin-releasing hormone (**GnRH**) secretion, inhibiting or delaying the preovulatory pulsatile peak release of LH and lowering plasma oestradiol-17β (Boland et al. 2001; Rhind et al. 1989), while increasing progesterone concentrations (Kiyma et al. 2004) can alter the endocrine function and interfere with the occurrence of typical/normal oestrous cycles (Houseknecht et al. 1998; Short and Bellows, 1999). The plasma glucose and insulin concentrations were similar in cows that had ACL and TCL supporting the evidence that ACL was not mediated by pituitary insulin signalling (Brothers et al. 2010; Sagae et al. 2012), which can affect oocyte development (Purcell and Moley, 2011; Yang et al. 2012) through leptin concentrations, which inhibit ovarian steroidogenesis (Spicer, 2001; Kendall et al. 2004) due to lower GnRH, gonadotropin (Yu et al. 1997; McCann et al. 1998) estradiol secretion (Spicer and Francisco, 1997). However, this lower pp DMI during the first 14 d pp in cows that had ACL may indicate an opportunity to investigate opportunities to enhance DMI through the management of cows, use of feeds, and feeding during early lactation. This may be achieved by improving pp DMI during the first 14 d pp by optimization of diet composition in relation to the average requirements of the dairy cows in the feeding group to achieve a higher DMI (Fish et al. 2012; Llonch et al. 2018; Rumphorst et al. 2022). The inclusion of rumen-protected choline and dry propylene glycol in dairy cow diets during the pre and pp transitions period

(Chung et al. 2009). Moreover, making changes to animal management much earlier in the life of dairy heifers by applying group rearing of calves, which improves calf feed intake, health and survival (Costa et al. 2015 and 2016) but more importantly increases the resilience of dairy cattle to regrouping, especially around first parturition (Zhang et al. 2022). While the genetic selection of cows for fertility traits in addition to MY and FE continues to improve the PR and fertility of dairy cows (Pryce et al., 2001; Lucy, 2019; Brito et al., 2021).

The MY of dairy cows has been associated with a longer PPI to ovarian cyclicity and inconsistent oestrous cycle lengths (Lucy, 2003; Friggens and Chagunda, 2005). The MY and ECM, FPCM and milk solids yield in Experiment 2 were and MY in Experiment 4 tended to be greater, but the ECM, FPCM and solids yield were not greater in Experiments 3 and 4. In previous studies, greater MY per cow has been associated with a decline in fertility (FAO, 2005; Butler, 2003; Royal et al. 2000; Moore and Thatcher, 2006; Bello et al. 2012) and longer PPI to ovarian cyclicity and inconsistent oestrous cycle lengths (Lucy, 2003; Friggens and Chagunda, 2005). While cows with ACL had prolonged PPI to P4 rise and commencement of ovarian activity, mean milk yield was not consistently greater in the studies in this thesis. However, on closer inspection showed that MY was greater in the first week pp in cows that had ACL in Experiments 2, 3 and 4 . This effect of rapidly accelerating MY following parturition has been noted by researchers previously and was found to result in considerable metabolic and physiological stressors (Domecq et al. 1997; Ingvarsten et al. 2003; Hansen et al. 2005) extending the PPI to conception, compared with a relatively flat and more steeply ascending lactation curve that reach peak MY and resumed pp ovarian activity sooner (Andersen et al. 2011). This approach to lowering the effect of ACL and prolonged PPI to P4 rise and resumption of ovarian activity is one that is more long term, but equally essential in maintaining a regular calving frequency for dairy cows. The effect of the ACL and prolonged PPI to first P4 rise and resumption of ovarian activity on CR and PR was more variable. The CR was low at 130 d pp and had improved by 160 d pp in most studies, with the exception of Exp. 4. It has been shown that lower CRs to first have been found when cows receive insemination < 60 d pp (Inchaisiri et al. 2010).

6.6: Calving interval and replacement rate related carbon footprint

The insemination of cows in the studies in this thesis typically occurred following peak lactation, which in practical terms, this has led to a delay in first insemination from > 50 d pp to > 60 d pp for higher MY cows (Andersen et al. 2011) and while the PPI to conception did not differ between cows that had ACL and TCL in the majority of the studies in this thesis, the PPI to conception was frequently greater than 80 d pp and up to 129 d and the CI were above 365 d and closer to and beyond 375 d in most experiments, up to a maximum of 412 d pp.

This is in keeping with previous studies that have found that CI has progressively increased (Hare et al. 2006) and the average CI for Holstein–Friesian cows in the UK has been reported to be 428 d (75th quartile 463 and 90th decile 546 d) (Gates 2013). This is greater than those in the studies in this thesis. Greater GHG emissions related to a longer CI and higher RR (Bell and Wilson, 2018) and the rearing of more replacement animals (Zehetmeier et al. 2014; Bell et al. 2015), which was associated with 14.9 kg/d of additional CI over 365 d. The CI achieved by cows in this this were consistently above 365 d, especially in cows that had ACL. The greater CI and especially CI related RR and RR related to PR resulted in the production of considerable amounts of CO2-eq. in the studies included in this thesis. The data from this study supports the findings of previous modelling (Garnsworthy, 2004; Bell and Wilson, 2018), demonstrating that substantial reduction in CO2-eq. related to lowering the CI and RR could be achieved by improvements in CI and RR, potentially by pp resumption of ovarian activity. This may be achieved by the continued selective breeding of cows with lower rise to and peak milk yields and in the shorter-term by improving pp DMI during the first 14 d pp through the management of the early lactation transition period (1 to 3 weeks pp), The inclusion of rumen-protected choline and dry propylene glycol in cows diets during the pre and pp transitions period (Chung et al. 2009) and optimization of diet composition in relation to the average requirements of the dairy cows in the feeding group to achieve a high levels of DMI (Fish et al. 2012; Llonch et al. 2018; Rumphorst et al. 2022). This period is characterized by regrouping of cows and group rearing of calves during early life, which not only improves calf feed intake, health and survival (Costa et al. 2015 and 2016) but more importantly increases the resilience of dairy cattle to regrouping around parturition (Zhang et al. 2022), which has the potential to increase DMI in the first few weeks following partition.

6.7: Outcomes in relation to the objectives of the thesis

The objectives of this thesis were to use milk P4 concentrations to evaluate the occurrence rate of ACL, which occurred in approx. 30 to 44 % of high MY cows. The effect of ACL on the fertility was mainly the increase in the PPI to first insemination and conception, with relatively low PR being achieved at 130 d pp. This extended the CI and increased RR, these increase CO_2-eq. partly due to an extended the CI, but mostly due to the effect of RR, which substantially increased the number of replacements required and CO_2-eq. of milk production.

This thesis looked at relatively high yielding Holstein Friesian dairy cows and compared cows that had ACL and TCL in terms of milk yield and FE, which while cows that had ACL in Expt. 2 had higher MY, however, this study had a limited number of cows and led to existing data sets being combined in Experiment 3 and 4. The MY of cows with ACL and TCL did not differ in Experiment 3, which contraindicated Expt. 2 and warranted further assessment with another

larger data set, which showed that MY tended to be greater in cows that had ACL than TCL in Expt. 4. The strength of the R correlation values between ovarian cycle length and fertility, FE, and the feed, CI and RR, FPCM, BCSP, pp BCS change, and CO2 eq. showed little clear evidence of specific factors that directly related to cycle length. However, in Expt. 2 to 4 the assessment of BCS clearly showed that cows calved at industry target BCS and there was little pp change in BCS in cows with ACL and TCL and no correlation between cycle length and BCS, showing that NEB was not a factor related to ACL. The assessment of plasma NEFA and BHB in experiment 4 supported this, while plasma insulin and glucose concentrations (Expt. 4) showed no difference between cows with ACL and TCL and little correlation with cycle length.

The thesis compared the PPI to conception and related calculated CI, and PR related and additional days of CI related RR and need for herd replacements and showed that RR increased the CO_2 eq. to a greater extent than additional days of CI at 130 d and that PR increased and RR reduced at 160 d pp. The evaluation of ACL and fertility of dairy cows showed that CO2-eq. emissions could be lowered by reducing herd RR by increasing PR by extending the service period from up to 130 to up to 160 d pp, to increase PR, despite the increase in additional days over a CI of 365 d, which had a much lower impact on CO2-eq. emissions than low PR.

6.8: Study limitations and difficulties

Work on dairy cow fertility requires working with large numbers of cows and the collection of all the data for this experiment could not be completed within the time and resources available during the time available for the completion of a PhD, between 2017 and 2022 especially due to the limitations encountered when facilities such as the farm and labs were closed due to COVID 19. The collection of milk samples thrice weekly and cost of progesterone concentrations remain labour intensive and costly. The application of technological developments and precision farming using automated P4 assessment using on farm cow side testing may offer the opportunity to evaluate the return to ovarian activity of dairy cows as a method of evaluating how cow management, especially early lactation (up to 100 d pp) nutrition. The collection and use of individual animal feed intake was essential for the evaluation of the factors related to early pp factors associated with early pp fertility and this data has to be collected where facilities make this possible. Changes in the supervisory team presented some difficulty during the PhD, however my second main supervisor was familiar to me as my previous internal assessor and supported me throughout the completion of this PhD following the retirement of my first main supervisor and I would like to thank Dr G Mann for the initial PhD opportunity.

References

References

Abdel, M. A, Emad, S, Gohary, E. L, Gabr, A. A, Ahmed, S. A, Ebrahim, S. A, et al. (2016). Impact of supplementing propylene glycol and calcium propionate to primiparous buffalo cows during the late gestation and early lactation period on reproductive performance and metabolic parameters. Alexandria Journal of Veterinary Sciences, 51(1): 114-121.

Adamczyk, K., Makulska, J., Jagusiak, W. and Węglarz, A. (2017). Associations between strain, herd size, age at first calving, culling reason and lifetime performance characteristics in Holstein-Friesian cows. Animal, 11(2):327–34.

Abdullah, F., Chung, E. L, Abba, Y., Tijjani, A., Sadiq, M. A., Mohammed, K., et al. (2015). Management of clinical case of endometritis in a cow: A case report. Journal Veterinary Advances, 5(4): 887-890.

Acosta-Martinez, M. (2011). PI3K: an attractive candidate for the central integration of metabolism and reproduction. Frontiers in Endocrinology, 2, 110.

Adamiak, S. J., K. Mackie, R. G. Watt, R. Webb, and K. D. Sinclair. (2005). Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biology of Reproduction, 73 (5) 918–926.

Adam, C. L., P. A. Findlay, and A. H. Moore (1998). Effects of insulin-like growth factor-1 on luteinizing hormone secretion in sheep. Animal Reproduction Science, vol. 50, no. 1-2, pp. 45–56.

Adamiak, S. J., Mackie, K., Watt, R. G., Webb, R. and Sinclair, K. D. (2005). Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biology of Reproduction, 73, 918-26.

Adriaens, I., Huybrechts, T., Geerinckx, K., Daems, D., Lammertyn, J., De Ketelaere, B., Aernouts, B. (2017). Mathematical characterization of the milk progesterone profile as a leg up to individualized monitoring of reproduction status in dairy cows. Theriogenology. https://doi.org/10.1016/j.theriogenology.2017.07.040.

Adriaens, I., Saeys, W., Huybrechts, T., Lamberigts, C., François, L., Geerinckx, K., Leroy, J., De Ketelaere, B. and Aernouts, B (2018). A novel system for on-farm fertility monitoring based on milk progesterone. Journal of Dairy Science, 101 (9). 8369-8382.

Aguilar-Pérez, C., Ku-Vera, J., Centurión-Castro, F. and Garnsworthy, P. C. (2009). Energy balance, milk production and reproduction in grazing crossbred cows in the tropics with and without cereal supplementation. Livestock Science, 122, 227-233.

AHDB (2022). Average UK milk yields by milk year. https://ahdb.org.uk/dairy/uk-milk-yield. Accessed 15.30, 15.12/2022.

AHDB (2022). All year round calving key performance indicators for dairy production, https://ahdb.org.uk/all-year-round-calving-key-performance-indicators accessed 12.52 GMT 10/07/22

AHDB (2020). Body condition scoring (BCS) https://ahdb.org.uk/knowledge-library/body-condition-scoring-flow-chart (Accessed 16.30 GMT 10/07/22).

AHDB (2019). The InCalf Guide for farmers calving all year round. Available at: https://projectblue.blob.core.windows.net/media/Default/Dairy/Publications/InCalfAYR2296_210128_WEB.pdf (Accessed: 6 April 2022).

Allen Garverick, H., and Smith, M. F. (2016). Female Reproductive Physiology and Endocrinology of Cattle. Veterinary Clinics of North America: Food Animal Practice. https://doi.org/10.1016/s0749-0720(15)30643-5.

Alexander, B. M., Z. Kiyma, M. McFarland et al. (2007). Influence of short-term fasting during the luteal phase of the estrous cycle on ovarian follicular development during the ensuing proestrus of ewes. Animal Reproduction Science, 97 (3-4), 356–363.

American-Diabetes-Association (2019). Lifestyle management: Standards of medical care in diabetes. Diabetes Care. (2019) 42:S46–60. doi: 10.2337/dc19-S005.

Anthony, R. V., Bellows, R. A., Short, R. E., Staigmiller, R. B., Kaltenbach, C. C., Dunn, T. G. (1986). Fetal growth of beef calves. I. Effect of prepartum dietary crude protein on birth weight, blood metabolites and steroid hormone concentrations. Journal of Animal Science, Sci. 62, 1363–1374.

Antoniazzi A. Q, Webb B. T, Romero J. J, Ashley R. L, Smirnova N. P, Henkes L. E, Bott R. C, Oliveira J. F, Niswender G. D, Bazer F. W, Hansen T. R (2013) Endocrine delivery of interferon-τ protects the corpus luteum from prostaglandin F2a-induced luteolysis in ewes. Biology of Reproduction, 88:1–12.

AOAC (1990) Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemist, Washington DC.

Arosh J. A, Banu S. K, McCracken J. A (2015) Novel concepts on the role of prostaglandins on luteal maintenance and maternal recognition and establishment of pregnancy in ruminants. Journal Dairy Science, 99:5926–5940.

Armstrong, D. G., J. G. Gong, and R. Webb (2003). Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. Reproduction Supplement, vol. 61, pp. 403–414, 2003.

Arundhat, O. I, Rahawy, M. A, Hadad, J. J. (2015). Bacterial isolates associated with dystocia and Retained placenta in Iraqi buffaloes. Reproduction of Domestic Animals, 43: 286-292.

Aschenbach, J. R.; Kristensen, N. B.; Donkin, S. S.; Hammon, H. M.; Penner, G. B. (2010). Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. IUBMB Life, 62, 869–877.

Ashworth, C. J., Toma, L. M. and Hunter, M.G. (2009). Nutritional effects on oocyte and embryo development in mammals: implications for reproductive efficiency and environmental sustainability. Philosophical Transactions of the Royal Society Biological Sciences, 364, 3351-3361.

Atashi, H., Asaadi, A. and Hostens, M. (2021). Association between age at first calving and lactation performance, lactation curve, calving interval, calf birth weight, and dystocia in Holstein dairy cows. PLoS One. 2021 Jan 4; 16(1): e0244825. doi: 10.1371/journal.pone.0244825. PMID: 33395420; PMCID: PMC7781378.

Aungier, SPM., J. F. Roche, P. Duffy, S. Scully, M. A. Crowe (2015). The relationship between activity clusters detected by an automatic activity monitor and endocrine changes during the periestrous period in lactating dairy cows. Journal of Dairy Science, 98 (2015), pp. 1666-1684.

Bach, A. (2011). Strategies to improve the economic sustainability of breeding of dairy cattle. Large Animal Review, 17, 209-216.

Banos, G., Brotherstone, S. and Coffey, M. P. (2007). Prenatal maternal effects on body condition score, female fertility, and milk yield of dairy cows. Journal of Dairy Science, 90, 3490-3499.

Barb, C.R., Barrett, J.B., Kraeling, R.R., Rampacek, G. B. (1999). Role of leptin in modulating neuroendocrine function: a metabolic link between the brain-pituitary and adipose tissue. Reproduction in Domestic Animals, 34 (3-4):111–125.

Bauman, D. E.; Griinari, J. M. (2003). Nutritional regulation of milk fat synthesis. Annual Review of Nutrition, 23, 203–227.

Beam, S. W., and Butler, W. R. (1999). Effects of energy balance on follicular development and first ovulation in postpartum. Reproduction and Fertility, Supplement 54, 411–424.

Bedere, N., L. Delaby, V. Ducrocq, S. Leurent-Colette, C. Disenhaus (2016). Toward improved postpartum cyclicity of primiparous dairy cows: Effects of genetic merit for production traits under contrasting feeding systems. Journal of Dairy Science, 99, (2), 1266-1276.

Bell, A. (1995). Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science, 73: 2804-2819.

Bell, M. J., Garnsworthy, P. C., Stott, A. W., and Pryce, J. E. (2015). Effects of changing cow production and fitness traits on profit and greenhouse gas emissions of UK dairy systems. The Journal of Agricultural Science, 153(1), 138-151.

Bell, M. J., M., Maak, M., Sorley and R. Proud (2018). Comparison of methods for monitoring the body condition of dairy cows. Front. Sustain. Food Syst., 26 November 2018. Sec.

Agroecology and Ecosystem Services. https://doi.org/10.3389/fsufs.2018.00080. Accessed 16/07/2022, 10.38.

Bell, M. J. and Wilson, P. (2018). Estimated differences in economic and environmental performance of forage-based dairy herds across the UK. Food and Energy Security, 7(1), e00127.

Bello, N. M., Stevenson, J. S. and Tempelman, R. J. (2012). Invited review: milk production and reproductive performance: modern interdisciplinary insights into an enduring axiom. Journal of Dairy Science, 95, 5461-5475.

Bellows, R. A., Short, R. E., (1978). Effects of precalving feed level on birth weight, calving difficulty and subsequent fertility. Journal of Animal Science, 46, 1522–1528.

Berger, P. J., Shanks, R. D., Freeman, A. E., and Laben, R. C. (1981). Genetic aspects of milk yield and reproductive performance. Journal of Dairy Science, 64(1), 114–122.

Berthiaume, R., Dubreuil, P., Stevenson, M., McBride, B. W., Lapierre, H. (2001). Intestinal Disappearance and Mesenteric and Portal Appearance of Amino Acids in Dairy Cows Fed Ruminally Protected Methionine. Journal of Animal Science, 84, 194–203.

Boland M. P. and P. Lonergan (2005). Effects of nutrition on fertility in dairy cows. Advanced Dairy Technology, 15, 19–33.

Boland, M. P., P. Lonergan, and D. O'Callaghan (2001). Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology, 55 (6) 1323–1340.

Boulton, A. C, Rushton, J. and Wathes, D. C. (2015). A study of dairy heifer rearing practices from birth to weaning and their associated costs on UK dairy farms. Open Journal of Animal Sciences. 5:185–97.

Borowczyk, E., J. S. Caton, D. A. Redmer et al. (2006). Effects of plane of nutrition on in vitro fertilization and early embryonic development in sheep. Journal of Animal Science, vol. 84, no. 6, pp. 1593–1599.

Borwick, S. C., S. M. Rhind, S. R. McMillen, and P. A. Racey (1997). Effect of undernutrition of ewes from the time of mating on fetal ovarian development in mid gestation. Reproduction, Fertility and Development, 9 (7) 711–715, 1997.

Boyd, G.W., Kiser, T.E., Lowrey, R.S. (1987). Effects of prepartum energy intake on steroids during late gestation and on cow and calf performance. Journal of Animal Science. 64, 1703–1709.

Brothers, K. J., S. Wu, S. A. Divall et al. (2010). Rescue of obesity-induced infertility in female mice due to a pituitary-specific knockout of the insulin receptor. Cell Metabolism. 12 (3) 295–305.

Bisinotto, R. S., L. F. Greco, E. S. Ribeiro, N. Martinez, F. S. Lima, C. R. Staples, W. W. Thatcher, J. E. P. Santos (2012). Influences of nutrition and metabolism on fertility of dairy cows. Animal Reproduction, 9, (3), 260-272.

Brickell, J. S., Bourne, N., McGowan, M. M., Wathes, D. C. (2009). Effect of growth and development during the rearing period on the subsequent fertility of nulliparous Holstein-Friesian heifers. Theriogenology. 72: 408–416.

Brito, L. F. H. R. Oliveira, K. Houlahan, P. A. S. Fonseca, S. Lam, A. M. Butty, D. J. Seymour, G. Vargas, T. C. S. Chud, F. F. Silva, C. F. Baes, A. Canovas, F. Miglior, F. S. Schenkel (2020). Invited Review: Genetic mechanisms underlying feed utilization and implementation of genomic selection for improved feed efficiency in dairy cattle. Canadian Journal of Animal Science, 100, 587-604.

Brito, L.F., N. Bedere, F. Douhard, H.R. Oliveira, M. Arnal, F. Peñagaricano, A.P. Schinckel, C.F. Baes, F. Miglior (2021). Review: Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. Animal, 15 (1) 100292.

Bruinjé, T. C., Colazo, M. G., Ribeiro, E. S., Gobikrushanth, M., and Ambrose, D. J. (2019). Using in-line milk progesterone data to characterize parameters of luteal activity and their association with fertility in Holstein cows. Journal of Dairy Science, 102(1), 780–798.

Bucholtz, D. C., N. M. Vidwans, C. G. Herbosa, K. K. Schillo, and D. L. Foster, Metabolic interfaces between growth and reproduction. V. Pulsatile luteinizing hormone secretion is dependent on glucose availability. Endocrinology, vol. 137, no. 2, pp. 601–607, 1996.

Buckley F., Dillon P., Rath M., Veerkamp R. (2000). The relationship between genetic merit for yield and live weight, condition score, and energy balance of spring calving Holstein Friesian dairy cows on grass-based systems of milk production. Journal of Dairy Science **83** (10984166): 1878-1886.

Bulman, D. C., and Lamming, G. E. (1979). The use of milk progesterone analysis in the study of oestrus detection, herd fertility and embryonic mortality in dairy cows. British Veterinary Journal, 135(6), 559–567.

Butler, W. R. (2000). Nutritional interactions with reproductive performance in dairy cattle. Animal Reproduction Science, 60, 449–457.

Butler, W. R. (2003) Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Prodouction Science 83: 211-218.

Butler, W. R. (2010). Review: Effect of Protein Nutrition on Ovarian and Uterine Physiology in Dairy Cattle. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(98)70146-8.

Butler, W. R., and Smith, R. D. (1989). Interrelationships between energy balance and pp reproductive function in dairy cattle. Journal of Dairy Science, 72(3), 767–783.

Café, L. M., Hennessy, D. W., Hearnshaw, H., Morris, S. T., Greenwood, P. L. (2006). Influences of nutrition during pregnancy and lactation on birth weights and growth to weaning of calves sired by Piedmontese and Wagyu bulls. Australian Journal of Experimental Agriculture. 46, 245–255.

Caro, D., Davis, S. J., Bastianoni, S., and Caldeira, K. (2014). Global and regional trends in greenhouse gas emissions from livestock. Climatic change, 126 (1), 203-216.

Cameron, J. L. and C. Nosbisch (1991). Suppression of pulsatile luteinizing hormone and testosterone secretion during short term food restriction in the adult male rhesus monkey (Macaca mulatta). Endocrinology, 128 (3) 1532–1540.

Campfield L. A, Smith F. J, Guisez Y., Devos R., Burn, P. (1995). Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 269 (5223):546–549.

Canfield, R. W., Sniffen, C.J. and Butler, W.R., 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. Journal of Animal Science, 73: 2342-2349.

Capper, J. L. and Bauman, D. E. (2013). The role of productivity in improving the environmental sustainability of ruminant production systems. Annual Review of Animal Bioscience, 1 (1), 469-489.

Capper, J. L., R.A. Cady, D.E. Bauman (2009). The environmental impact of dairy production: 1944 compared with 2007. Journal of Animal Science, 87, pp. 2160-2167.

Casanueva, F. F. and C. Dieguez (1999). Neuroendocrine regulation and actions of leptin. Frontiers in Neuroendocrinology, 20 (4) 317–363.

Cervero, A., Domínguez, F., Horcajadas, J. A., Quiñonero, A., Pellicer, A., Simón, C. (2006). The role of the leptin in reproduction. Current Opinion in Obstetrics and Gynecology. 18(3):297–303.

Chandrashekar, V., D. Zaczek, and A. Bartke (2004). The consequences of altered somatotropic system on reproduction. Biology of Reproduction, vol. 71, no. 1, pp. 17–27.

Chaffaux, S., Lakdissi, H., Thibier, M. (1991). Epidemiology of clinical metritis and endometritis. Record Medical Veterinary science. 1991; 167: 349-358.

Chagas, L. M., J. J. Bass, D. Blache et al. (2007). Invited review: new perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. Journal of Dairy Science, 90 (9) 4022–4032.

Chagas, L. M., [*]M.C.Lucy[†]P.J.Back[*]D.Blache[‡]J.M.Lee[*]P.J.S.Gore[*]A.J.Sheahan[*]J.R.Roche (2008). Insulin resistance in divergent strains of Holstein-Friesian dairy cows offered

fresh pasture and increasing amounts of concentrate in early lactation. Journal of Dairy Science, 92 (1), 216-222.

Chagas L. M., Rhodes F.M., Blache D., Gore P.J.S., Macdonald K.A., Verkerk G.A. (1989). Precalving feeding and body condition effects on metabolic responses and postpartum anestrus interval in grazing primiparous dairy cows. Journal of Dairy Science 2006; 89: 1981-1989.

Chagas, L. M., Bass, J. J., Blache, D., Burke, C. R., Kay, J. K., Lindsay, D. R., et al. (2007). Invited review: new perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. Journal of Dairy Science 90, 4022–4032.

Chebel, R. C., Demétrio, D. G. B., and Metzger, J. (2008). Factors affecting success of embryo collection and transfer in large dairy herds. Theriogenology, 69(1), 98–106.

Cheng, X. B, Wen JP, Yang J, Yang Y, Ning G, Li XY. (2011). GnRH secretion is inhibited by adiponectin through activation of AMP-activated protein kinase and extracellular signal-regulated kinase. Endocrine, 39 (1):6–12.

Chung, Y. H., Brown, N. E., Martinez, C. M., Cassidy, T. W. and Varga, G. A. (2009). Effects of rumen-protected choline and dry propylene glycol on feed intake and blood parameters for Holstein dairy cows in early lactation. Journal of Dairy Science, 92 (6), 2729-2736.

Clarke, L.J., and Henry, B.A. (1999). Leptin and reproduction. Reviews of Reproduction. 4 (1):48–55.

Clay, N., T. Garnett, J. Lorimer (2020). Dairy intensification: drivers, impacts and alternatives. Ambio, 49, pp. 35-48.

Cooke, J. S., Cheng, Z., Bourne, N. E., Wathes, D. C. (2013). Association between growth rates, age at first calving and subsequent fertility, milk production and survival in Holstein-Friesian heifers. Open Journal Animal Science. Scientific Research Publishing; 3: 1–12.

Costa, J. H. C., Von Keyserlingk, M. A. G., & Weary, D. M. (2016). Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. Journal of Dairy Science, 99(4), 2453-2467.

Costa, J. H. C., Meagher, R. K., Von Keyserlingk, M. A. G., & Weary, D. M. (2015). Early pair housing increases solid feed intake and weight gains in dairy calves. Journal of Dairy Science, 98(9), 6381-6386.

Cottle, D.J., J. V. Nolan and S.G. Wiedemann (2022). Ruminant enteric methane mitigation: A review. Animal Production Science. 51, 491–514.

Craig, J., Zhu H., Dyce, P. W., Petrik, J., Li, J. (2004). Leptin enhances oocyte nuclear and cytoplasmic maturation via the mitogen-activated protein kinase pathway. Endocrinology. 145 (11):5355–5363.

Craig JA, Zhu H, Dyce PW, Wen L, Li J. (2005). Leptin enhances porcine preimplantation embryo development in vitro. Molecular and Cellular Endocrinology. 229 (1-2):141–147.

Crowe, M. A. (2008). Fertility in dairy cows–the conference in perspective. Fertility in Dairy Cows–Bridging the Gaps' (Eds. M. D. Royal, R. F. Smith and N. C. Friggens) 175–179.

Crutzen, P. J., I. Aselmann and W. Seiler (1986). Methane production by domestic animals, wild ruminants, other herbivorous fauna, and humans, Tellus B: Chemical and Physical Meteorology, 38:3-4, 271-284. DOI: 10.3402/tellusb.v38i3-4.15135.

Cutullic, E., L. Delaby, Y. Gallard, C. Disenhaus (2011). Dairy cows' reproductive response to feeding level differs according to the reproductive stage and the breed. Animal, 5, 731-740.

Cutullic, E., L. Delaby, Y. Gallard, C. Disenhaus (2012). Towards a better understanding of the respective effects of milk yield and body condition dynamics on reproduction in Holstein dairy cows. Animal, 6, 476-487.

Cunningham MJ, Clifton DK, Steiner RA. (1999). Leptin's actions on the reproductive axis: perspectives and mechanisms. Biology of Reproduction. 60 (2):216–222.

Daftary, S. S. and A. C. Gore (2005). IGF-1 in the brain as a regulator of reproductive neuroendocrine function. Experimental Biology and Medicine, vol. 230, no. 5, pp. 292–306.

Dall-Orsoletta, A.C., M.M. Oziemblowski, A. Berndt, H.M.N. Ribeiro-Filho (2019). Enteric methane emission from grazing dairy cows receiving corn silage or ground corn supplementation. Animal Feed Science and Technology. 253, 65-73.

Dalton, J. C., Nadir, S., Bame, J. H., Noftsinger, M., Nebel, R. L., and Saacke, R. G. (2001). Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. Journal of Dairy Science, 84(11), 2413–2418.

Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al. (2018). Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. (2018) 61:2461–98. doi: 10.1007/s00125-018-4729-5.

de Garis P., Lean I., Rabiee A., Heuer C. (2010). Effects of increasing days of exposure to prepartum transition diets on reproduction and health in dairy cows. Australian Veterinary Journal, 88 (a): 84-92.

de Haas, Y., Pszczola, M., Soyeurt, H., Wall, E., and Lassen, J. (2017). Invited review: Phenotypes to genetically reduce greenhouse gas emissions in dairying. Journal of Dairy Science, 100 (2), 855-870.

de Veth M., Bauman D., Koch W., Mann G., Pfeiffer A., Butler W. (2009). Efficacy of conjugated linoleic acid for improving reproduction: A multi-study analysis in early-lactation dairy cows. Journal of Dairy Science 92: 2662-2669

De Vries, A. (2017). Economic trade-offs between genetic improvement and longevity in dairy cattle. Journal of Dairy Science, 100(5), 4184-4192.

Demeestere, I., C. Gervy, J. Centner, F. Devreker, Y. Englert, and A. Delbaere (2004). Effect of insulin-like growth factor-I during preantral follicular culture on steroidogenesis, in vitro oocyte maturation, and embryo development in mice. Biology of Reproduction, vol. 70, no. 6, pp. 1664–1669.

Deori. S. and Arundhati, P. (2015). Bovine postpartum metritis and its therapeutics: A review. Indian Journal of Science and Technology. 2015; 8(23): 45.

Dillon, P., Berry, D. P., Evans, R. D., Buckley, F., and Horan, B. (2006). Consequences of genetic selection for increased milk production in European seasonal pasture-based systems of milk production. Livestock Science, 99 (2–3), 141–158.

Diskin, M. G., D. R. Mackey, J. F. Roche, and J. M. Sreenan (2003). Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Animal Reproduction Science, vol. 78, no. 3-4, pp. 345–370, 2003.

Diskin, M. G., Morris, D. G. (2008) Embryonic and early foetal losses in cattle and other ruminants. Reproduction of Domestic Animals. 43:260–267.

Donaghy, A. J., and Baxter, R. C. (1996). Insulin-like growth factor bioactivity and its modification in growth hormone resistant states. Bailliere's Clinical Endocrinology and Metabolism. https://doi.org/10.1016/S0950-351X(96)80560-X.

Dorniak P, Bazer FW, Spencer TE (2011) Prostaglandins regulate conceptus elongation and mediate effects of interferon tau on the ovine uterine endometrium. Biology of Reproduction, 84:1119–1127.

Downing, J. A. J. Joss, and R. J. Scaramuzzi (1995). Ovulation rate and the concentrations of gonadotrophins and metabolic hormones in ewes infused with glucose during the late luteal phase of the oestrous cycle. Journal of Endocrinology, 146 (3) 403–410.

Drackley, J.K.; Dann, H.M.; Douglas, N.; Guretzky, N.A.J.; Litherland, N.B.; Underwood, J.P.; Loor, J.J. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science, 4, 323–344.

Drackley, J. K. and C. K. Reynolds (2021). The impact of improving feed efficiency on the environmental impact of livestock production. Burleigh Dodds Science Publishing

Limited, 2021. Baines, R. (ed.), Reducing greenhouse gas emissions from livestock production, Burleigh Dodds Science Publishing, Cambridge, UK, 2021, (ISBN: 978 1 78676 439 3; www.bdspublishing.com).

Du M, Tong J, Zhao J, Underwood KR, Zhu M, Ford SP, et al. (2010). Fetal programming of skeletal muscle development in ruminant animals. Journal Animal Science. 88:51–60.

Dupont, C., A. G. Cordier, C. Junien, B. Mandon-Pépin, R. Levy, and P. Chavatte-Palmer (2012). Maternal environment and the reproductive function of the offspring. Theriogenology, 78 (7) 1405–1414, 2012.

Eastham, N. T, Coates, A, Cripps, P., Richardson, H., Smith, R., Oikonomou, G. (2018). Associations between age at first calving and subsequent lactation performance in UK Holstein and Holstein-Friesian dairy cows. PLoS One. 2018;13(6):e0197764.

Eastridge, M. L. (2006). Major advances in applied dairy cattle nutrition. Journal of Dairy Science, 89, 1311–1323.

Edmonson, A.J., I.J. Lean, L. D. Weaver, T. Farver (1989). A body condition scoring chart for Holstein dairy cows. Journal of Dairy Science, 72 (1) 68-78.

Edwards, J. L., Ealy, A. D., Monterroso, V. H., and Hansen, P. J. (1997). Ontogeny of temperature-regulated heat shock protein 70 synthesis in preimplantation bovine embryos. Molecular Reproduction and Development: Incorporating Gamete Research, 48 (1), 25–33.

Eggar-Danner, C., Cole, J. B., Pryce, J. E., Gengler, N., Heringstad, B., Bradley, A., and Stock, K. F. (2015). Invited review: Overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. Animal, 9, 191–207.

El-Kadi, S. W., McLeod, K. R., Elam, N. A., Kitts, S. E., Taylor, C. C., Harmon, D. L., Bequette, B.J., Vanzant, E.S. (2008). Nutrient net absorption across the portal-drained viscera of forage-fed beef steers: Quantitative assessment and application to a nutritional prediction model. Journal of Animal Science. 86, 2277–2287.

Erickson, G. F., V. G. Garzo, and D. A. Magoffin (1989). Insulin-like growth factor-I regulates aromatase activity in human granulosa and granulosa luteal cells. Journal of Clinical Endocrinology and Metabolism, 69 (4) 716–724, 1989.

Evans, J. J. and G. M. Anderson (2012). Balancing ovulation and anovulation: integration of the reproductive and energy balance axes by neuropeptides. Human Reproduction Update, 18 (3), 313–332.

Fenwick, M. A., R. Fitzpatrick, D. A. Kenny et al. (2008). Interrelationships between negative energy balance (NEB) and IGF regulation in liver of lactating dairy cows. Domestic Animal Endocrinology, vol. 34, no. 1, pp. 31–44.

Fish, J.A.; DeVries, T.J. Short communication: Varying dietary dry matter concentration through water addition: Effect on nutrient intake and sorting of dairy cows in late lactation. Journal of Dairy Science 2012, 95, 850–855.

Fleming T., Velazquez M., Eckert J., Lucas E., Watkins A. (2012). Nutrition of females during the peri-conceptional period and effects on foetal programming and health of offspring. Animal Reproduction Science. 130:193–197.

Flysjö, A., Cederberg, C., Henriksson, M., and Ledgard, S. (2012). The interaction between milk and beef production and emissions from land use change–critical considerations in life cycle assessment and carbon footprint studies of milk. Journal of cleaner production, 28, 134-142.

Fodor I, Lang Z, Ózsvári L. (2020). Relationship of dairy heifer reproduction with survival to first calving, milk yield and culling risk in the first lactation. Asian-Australasian Journal of Animal Sciences. 33(8):1360 10.5713/ajas.19.0474.

Fonseca, F. A., Britt, J. H., McDaniel, B. T., Wilk, J. C., and Rakes, A. H. (2010). Reproductive Traits of Holsteins and Jerseys. Effects of Age, Milk Yield, and Clinical Abnormalities on Involution of Cervix and Uterus, Ovulation, Estrous Cycles, Detection of Estrus, Conception Rate, and Days Open. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(83)81910-9.

Food and Agriculture Organization (2005) The State of Food and Agriculture. Production year book, Food and Agriculture Organization of the United Nations, Rome, Italy. Pg No: 233.

Forar, A. L., Gay, J. M., and Hancock, D. D. (1995). The frequency of endemic fetal loss in dairy cattle: a review. Theriogenology, 43(6), 989–1000.

Forbes, D. J. (2000) Dairy Cow Longevity - Controlling Culling. To Improve Profit. Cattle Practice 8(3): 305-310.

Ford, S. P., Long, N. M. (2011). Evidence for similar changes in offspring phenotype following either maternal undernutrition or overnutrition: Potential impact on fetal epigenetic mechanisms. Reprod. Fertil. Dev. 24:105–111.

Freetly, H. C., Ferrell, C. L., Jenkins, T. G. (2000). Timing of realimentation of mature cows that were feed-restricted during pregnancy influences calf birth weights and growth rates. Journal of Animal Science. 78, 2790–2796.

Friggens, N. and Labouriau, R. (2010). Probability of pregnancy as affected by oestrus number and days to first oestrus in dairy cows of three breeds and parities. Animal Reproduction Science, 118, 155-162.

Friggens, N.C., Ridder, C., and Lovendahl, P. (2007). On the use of milk composition measures to predict the energy balance of dairy cows. Journal of Dairy Science, 90, 5453-5467.

Froidmont, E., Mayeres, P., Picron, P., Turlot, A., Planchon, V., Stilmant, D. (2013). Association between age at first calving, year and season of first calving and milk production in Holstein cows. Animal. 7: 665–72.

Gabler, M., Tozer, P., Heinrichs, A. (2000). Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. Journal of Dairy Science. 83 (5):1104–9.

Gerber, P., Vellinga, T., Opio, C., Henderson, B., and Steinfeld, H. (2010). Greenhouse gas emissions from the dairy sector. A life cycle assessment. Food and Agricultural Organization of the United Nations: Animal Production and Health Division, Rome, Italy.

Gaillard, C., M. Barbu, T. Sørensen, J. Sehested, H. Callesen, M. Vestergaard (2006). Milk yield and estrous behavior during eight consecutive estruses in Holstein cows fed standardized or high energy diets and grouped according to live weight changes in early lactation. Journal of Dairy Science, 99, 3134-3141.

Gaines, J. D., Thomas, C. B., and Eicker, S. (1993). The interestrous interval profile of a dairy herd: how useful is it? Veterinary Medicine.

Gardner, I. A., D. W. Hird, W. W. Utterback, C. Danaye-Elmi, B. R. Heron, K. H. Christiansen, Sischo, W. M. (1990). Mortality, morbidity, case-fatality, and culling rates for California dairy cattle as evaluated by the national animal health monitoring system, 1986–87. Preventive Veterinary Medicine. 8, (2–3),157-170.

Garcia-Garcia, R. M., (2012). Integrative Control of Energy Balance and Reproduction in Females, International Scholarly Research Notices, vol. 2012, Article ID 121389, 13 pages, 2012.https://doi.org/10.5402/2012/121389.

García, M. C., López, M., Alvarez, C. V., Casanueva, F., Tena-Sempere, M., Diéguez, C. (2007). Role of ghrelin in reproduction. Reproduction. 133(3):531–540.

Garnsworthy, P. C. (2004). The environmental impact of fertility in dairy cows: A modelling approach to predict methane and ammonia emissions. Animal Feed Science and Technology, 112, 211–223. https://doi.org/10.1016/j.anifeedsci.2003.10.011

Garnsworthy, P. C. (2006). Body condition score in dairy cows: targets for production and fertility. Recent advances in animal nutrition, 40, 61.

Garnsworthy, P. C., Craigon, J., Hernandez-Medrano, J. H., and Saunders, N. (2012). Variation among individual dairy cows in methane measurements made on farm during milking. Journal of Dairy Science, 95 (6), 3181-3189.

Garnsworthy, P. C., A. Lock, G. E. Mann, K. D. Sinclair, and R. Webb (2008a). Nutrition, metabolism, and fertility in dairy cows: 1. Dietary energy source and ovarian function. Journal of Dairy Science, vol. 91, no. 10, pp. 3814–3823.

Garnsworthy, P. C., Lock, A., Mann, G. E., Sinclair, K. D. and Webb, R., (2008b). Nutrition, metabolism, and fertility in dairy cows: 2. Dietary fatty acids and ovarian function Journal of Dairy Science. 91(10), 3824-3833.

Garnsworthy, P. C., Gong, J. G., Armstrong, D. G., Newbold, J. R., Marsden, M., Richards, S. E., Mann, G. E., Sinclair, K. D. and Webb, R., 2008c. Nutrition, Metabolism, And Fertility In Dairy Cows: 3. Amino Acids and Ovarian Function. Journal Of Dairy Science. 91(11), 4190-4197.

Garnsworthy, P. C., Sinclair, K. D. and Webb, R. (2008d). Integration of physiological mechanisms that influence fertility in dairy cows Animal. 2(8), 1144-1152.

Gautam, G., Nakao. M., Yusuf. K., Koike, K. (2009). Prevalence of endometritis during postpartum Period and its impact on subsequent reproductive performance in two Japanese dairy herds. Animal Reproduction Science. 2009; 116: 175-187.

Gonzalez, J. M, Camacho L. E., Ebarb, S. M, Swanson, K. C., Vonnahme, K. A., Stelzleni, A. M. et al. (2013). Realimentation of nutrient restricted pregnant beef cows supports compensatory fetal muscle growth. Journal Anim Sci.; 91: 4797–4806.

Gordon, I. (1999). Controlled reproduction in cattle and buffaloes. Acribia, SA.

Grimard, B., Freret, S., Chevallier, A., Pinto, A., Ponsart, C. and Humblot, P. (2006). Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. Animal reproduction science, 91(1-2), 31-44.

Gröhn, Y. T., and Rajala-Schultz, P. J. (2000). Epidemiology of reproductive performance in dairy cows. Animal Reproduction Science, 60, 605–614.

Grummer, R. R. (2007). Strategies to improve fertility of high yielding dairy farms: Management of the dry period. Theriogenology, 68 Suppl 1, S281-8. https://doi.org/10.1016/j.theriogenology.2007.04.031.

Gunn, R. G., D. A. Sim, and E. A. Hunter (1995). Effects of nutrition in-utero and in early-life on the subsequent lifetime reproductive-performance of Scottish blackface ewes in 2 management-systems. Animal Science, 60, 223–230.

Gwazdauskas, F. C., Wilcox, C. J., and Thatcher, W. W. (2010). Environmental and Managemental Factors Affecting Conception Rate in a Subtropical Climate. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(75)84523-1.

Habel, J., P. Chapoutot, C, Koch and A. Sundrum (2022). Estimation of Individual Glucose Reserves in High-Yielding Dairy Cows. Dairy, 3, 438–464.

Habel, J. and Sundrum, A. (2020). Mismatch of Glucose Allocation between Different Life Functions in the Transition Period of Dairy Cows. Animals 2020, 10, 1028.

Hageman, W. H., Shook G., Tyler W. (1991). Reproductive performance in genetic lines selected for high or average milk yield. Journal of Dairy Science 74 (1787206): 4366-4376.

Hardie, L. J., D. V. Rayner, S. Holmes, and P. Trayhurn (1996) Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochemical and Biophysical Research Communications, vol. 223, no. 3, pp. 660–665.

Hare, E, Norman HD, Wright JR. (2006). Survival rates and productive herd life of dairy cattle in the United States. Journal Dairy Sci., 89:3713–3720.

Haruna, I. L., Y. Li., U. J. Ekegbu, H. Amirpour-Najafabadi, H. Zhou, and J. G. H. Hickford (2020). Associations between the Bovine Myostatin Gene and Milk Fatty Acid Composition in New Zealand Holstein-Friesian × Jersey-Cross Cows. Animals (Basel). 10 (9): 1447.

Hattori, N., Saito T, Yagyu T, Jiang BH, Kitagawa K, Inagaki C. GH, GH receptor, GH secretagogue receptor, and Ghrelin expression in human T cells, B cells, and neutrophils. Journal of Clinical Endocrinology and Metabolism. 2001;86(9):4284–4291.

Heinrichs, A. J. (1993). Raising dairy replacements to meet the needs of the 21st century. Journal of Dairy Science. 76 (10):3179–87.

Hidekitamura, J. K., Shimizu, T., Ishii, S., Sugihara, H., Wakabayashi, I. (2000). Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology. 141(12):4797–4800.

Hocquette, J. F. (2010). Endocrine and metabolic regulation of muscle growth and body composition in cattle. Animal, 4 (11) 1797–1809.

Hoedemaker, M., Prange, D. amd Gundelach, Y. (2009). Body condition change ante-and postpartum, health and reproductive performance in German Holstein cows. Reproduction in Domestic Animals, 44(2), 167–173.

Hoffman, P. (1997). Optimum body size of Holstein replacement heifers. Journal of Animal Science. 1997;75(3):836–45.

Horan, B., J. F. Mee, M. Rath, P. O'Connor, P. Dillon (2004). The effect of strain of Holstein-Friesian cow and feeding system on reproductive performance in seasonal-calving milk production systems. Animal Science. 79, 453-467.

Horan, B., Mee J., O'connor P., Rath M., Dillon P. (2005). The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. Theriogenology. 63 (15629811): 950-971.

Horn, M., Knaus, W., Kirner, L., and Steinwidder, A. (2012). Economic evaluation of longevity in organic dairy cows. Organic Agriculture, 2 (2), 127-143.

Hough, R. L., McCarthy, F. D., Kent, H. D., Eversole, D. E., Wahlberg, M. L. (1990). Influence of nutritional restriction during late gestation on production measures and passive immunity in beef cattle. Journal of Animal Science. 68:2622–2627.

Houseknecht, K. L., D. L. Boggs, D. R. Campion et al. (1998). Effect of dietary energy source and level on serum growth hormone, insulin-like growth factor 1, growth and body composition in beef heifers. Journal of Animal Science, 66 (11) 2916–2923.

Hubbert, W. T. (1972). Recommendations for standardizing bovine reproductive terms by committee on bovine reproductive nomenclature. Cornell Veterinary, 62(2), 216–237.

Hunter, M. G., R. S. Robinson, G. E. Mann, and R. Webb (2004). Endocrine and paracrine control of follicular development and ovulation rate in farm species. Animal Reproduction Science, 82-83, 461–477.

Huntington, G. B.; Harmon, D. L.; Richards, C. J. (2006). Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. Journal of Animal Science. 2006, 84, E14–E24.

Hurnik, J. F., King, G. J., and Robertson, H. A. (1975). Estrous and related behaviour in pp Holstein cows. Applied Animal Ethology. https://doi.org/10.1016/0304-3762(75)90065-6.

Hyttel, P., J. Laurincik, D. Viuff et al. (2000). Activation of ribosomal RNA genes in preimplantation cattle and swine embryos. Animal Reproduction Science, 60, 49–60.

Iain, M. S and Sian, E. O. (2017). Postpartum uterine infection and endometritis in dairy cattle. Paper presente at: The 33rd Annual Scientific Meeting of the European Embryo Transfer Association (AETE); Bath, United Kingdom.

IDF (2015). A Common Carbon Footprint Approach for the Dairy Sector, the IDF Guide to Life Cycle Assessment Methodology, Bulletin of the International Dairy Federation 479/2015. International Dairy Federation. Available online at: https://store.fil-idf.org/product/a-common-carbon-footprint-approach-for-the-dairy-sector-the-idf-guide-to-standard-life-cycle-assessment-methodology/ (accessed February 15, 2021).

Israel DD, Sheffer-Babila S, de Luca C, et al. (2012). Effects of leptin and melanocortin signaling interactions on pubertal development and reproduction. Endocrinology. 153:2408–2419.

Iwasa, T., T. Matsuzaki, M. Murakami et al. (2010). Sensitivities of mRNA expression levels of Kiss1 and its receptor, Kiss1r, to nutritional status are changed during the developmental period in female rats. Journal of Endocrinology, 207 (2), 195–202.

Jiao, H. P., Dale, A. J., Carson, A. F., Murray, S., Gordon, A. W., & Ferris, C. P. (2014). Effect of concentrate feed level on methane emissions from grazing dairy cows. Journal of Dairy Science. 97 (11), 7043-7053.

Jammes, H., C. Junien, and P. Chavatte-Palmer (2011). Epigenetic control of development and expression of quantitative traits. Reproduction, Fertility and Development, 23 (1) 64–74.

Jones, J. I., and Clemmons, D. R. (1995). Insulin-like growth factors and their binding proteins: Biological actions. Endocrine Reviews. https://doi.org/10.1210/edrv-16-1-3.

Jorritsma, R., M. W. De Groot, P. L. A. M. Vos, T. A. M. Kruip, T. Wensing, and J. P. T. M. Noordhuizen (2003). Acute fasting in heifers as a model for assessing the relationship between plasma and follicular fluid NEFA concentrations. Theriogenology, vol. 60, no. 1, pp. 151–161.

Kafi, M., A. Mirzaei, A. Tamadon, M. Saeb (2012). Factors affecting the occurrence of postpartum prolonged luteal activity in clinically healthy high-producing dairy cows. Theriogenology, 77 , 421-429.

Kastelic, J. P., and Ginther, O. J. (1991). Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. Animal Reproduction Science, 26(1–2), 13–24.

Kasimanickam, R., Kasimanickam, V., Kastelic, J. P. (2014). Mucin and cytokines mRNA in endometrium of dairy cows with postpartum uterine disease or repeat breeding. Theriogenology; 81: 952-958.

Kendall, N. R., Gutierrez, C. G., Scaramuzzi, R. J., Baird, D. T., Webb, R., Campbell, B. K. (2004). Direct in vivo effects of leptin on ovarian steroidogenesis in sheep. Reproduction. 128 (6):757–765.

Kenny, D. A., J. Heslin, and C. J. Byrne (2017). Early onset of puberty in cattle: implications for gamete quality and embryo survival. Reproduction, Fertility and Development 30(1) 101-117.

Kim, J. J. and D. Accili (2002). Signalling through IGF-I and insulin receptors: where is the specificity? Growth Hormone and IGF Research, vol. 12, no. 2, pp. 84–90.

Kiyma, Z., B. M. Alexander, E. A. Van Kirk, W. J. Murdoch, D. M. Hallford, and G. E. Moss (2004). Effects of feed restriction on reproductive and metabolic hormones in ewes. Journal of Animal Science, 82 (9) 2548–2557.

Kleen, J. L, Hooijer, G. A, Rehage, J and Noordhuizen, J. P. (2003). Subacute ruminal acidosis (SARA): a review. Journal of Veterinary Medicine. Series A: Physiology, Pathology, Clinical Medicine 50, 406–414.

Knapp, J.R., Laur, G. L., Vadas, P.A., Weiss, W. P. and Tricarico, J. M. (2014). Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions, Journal of Dairy Science 97(6), 3231–3261.

Knaus, W. (2009). Dairy cows trapped between performance demands and adaptability. Journal of the Science of Food and Agriculture, 89(7), 1107-1114.

Konigsson K, Gustafsson H, Kindah H. (2002). Ketodihdro-PGF2α, progesterone and uterine involution in primiparous cows with induced retained placenta and post partial endometritis. Reproduction of Domesticated Animals. 37: 43-51.

Korbonits, M., Goldstone, A. P, Gueorguiev, M., Grossman A. B. (2004). Ghrelin—a hormone with multiple functions. Frontiers in Neuroendocrinology. 2004;25(1):27–68.

Kotsampasi, B., S. Chadio, G. Papadomichelakis et al. (2009). Effects of maternal undernutrition on the hypothalamic-pituitary-gonadal axis function in female sheep offspring. Reproduction in Domestic Animals, 44 (4) 677–684.

Kristensen, N. B. and Harmon, D. L. (2004). Splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers 1. J. Journal of Animal Science. 82, 2033–2042.

Kumawat, B. L.,S. K. Singh, M. K. Patra, H. Kumar and N. Krishnaswamy (2022). New insights into the mechanism of maternal recognition of pregnancy in ruminants. Current Concepts in Bovine Reproduction, 107–118.

Kunaka, K., and Makuza, S. M. (2005). Genetic and environmental trends for milk traits for Zimbabwean Holstein-Friesian population. Pakistan Journal of Biological Sciences, 8(7), 1011–1015.

Lamming, G. E., and Darwash, A. O. (1998). The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. Animal Reproduction Science, 52 (3), 175–190.

Lapierre, H., Galindo, C., Lemosquet, S., Ortigues-Marty, I., Doepel, L., Ouellet, D. (2010). Protein supply, glucose kinetics and milk yield in dairy cows. In Energy and Protein Metabolism and Nutrition, 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Parma, Italy, 6–10 September 2010; Wageningen Academic Publishers: Wageningen; The Netherland, 2010; pp. 275–286. ISBN 9789086861538.

Lapierre, H. and Lobley, G. E. (2001). Nitrogen Recycling in the Ruminant: A Review. Journal of Dairy Science 2001, 84, E223–E236.

Lassen, J. and Difford, G. F. (2020). Genetic and genomic selection as a methane mitigation strategy in dairy cattle. Animal, 14, s473-s483.

Larsen, M. and Kristensen, N. B. (2009). Effect of abomasal glucose infusion on splanchnic and whole-body glucose metabolism in periparturient dairy cows. Journal of Dairy Science 2009, 92, 1071–1083.

Larsen, M. and Kristensen, N. B. (2013). Precursors for liver gluconeogenesis in periparturient dairy cows. Anim. Int. J. Anim. Biosci., 7, 1640–1650.

Laster, D. B. (1974). Factors affecting pelvic size and dystocia in beef cattle. Journal of Animal Science. 38 (3), 496–500.

Lean, I. J., Celi, P., Raadsma, H., McNamara, J., Rabiee, A. R. (2012). Effects of dietary crude protein on fertility: Meta-analysis and meta-regression. Animal Feed Sci. Technol. 171: 31-42.

LeBlanc, S. J, Duffield, T. F, Leslie, K. E, Batean, K. G, Keefe, G. P, Walton, J. S, et al. (2002). Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. Journal Dairy Sci. 2002; 85: 2223-2236.

Lee, J., McCracken JA, Stanley JA, Nithy TK, Banu SK, Arosh JA (2012) Intraluteal prostaglandin biosynthesis and signaling are selectively directed towards PGF2alpha during luteolysis but towards PGE2 during the establishment of pregnancy in sheep. Biology of Reproduction. 87:97.

Leppens-Luisier, G., F. Urner, and D. Sakkas, (2001). Facilitated glucose transporters play a crucial role throughout mouse preimplantation embryo development, Human Reproduction, vol. 16, no. 6, pp. 1229–1236.

Leroy, J. L., Van Hoeck, V., Clemente, M., Rizos, D., Gutierrez-Adan, A., Van Soom, A., Uytterhoeven, M., and Bols, P. E. (2010). The effect of nutritionally induced hyperlipidaemia on in vitro bovine embryo quality. Human Reproduction, 25, 768–778.

Linzell, J. L. (1972). Mechanism of Secretion of the Aqueous Phase of Milk. Journal of Dairy Science. 55, 1316–1322.

Liu, H.; Zhao, K.; Liu, J. (2013). Effects of glucose availability on expression of the key genes involved in synthesis of milk fat, lactose and glucose metabolism in bovine mammary epithelial cells. PLoS ONE 2013, 8, e66092.

Llewellyn, S., R. Fitzpatrick, D. A. Kenny, J. J. Murphy, R. J. Scaramuzzi, and D. C. Wathes (2007). Effect of negative energy balance on the insulin-like growth factor system in pre-recruitment ovarian follicles of post-partum dairy cows. Reproduction, vol. 133, no. 3, pp. 627–639.

Llonch, P.; Mainau, E.; Ipharraguerre, I.R.; Bargo, F.; Tedó, G.; Blanch, M.; Manteca, X. Chicken or the Egg: The Reciprocal Association Between Feeding Behavior and Animal Welfare and Their Impact on Productivity in Dairy Cows. Front. Veterinary Sci. 2018, 5, 305.

Lockyer, D. R. (1997). Methane emissions from grazing sheep and calves. Agricultural Ecosystems and Environment. 66:11–18.

Long, N. M., Tousley C. B., Underwood, K. R., Paisley, S. I., Means, W. J., Hess, B. W., Du, M., Ford, S. P. (2012). Effects of early- to mid-gestational undernutrition with or without protein supplementation on offspring growth, carcass characteristics, and adipocyte size in beef cattle. Journal of Animal Science. 90:197–206. doi: 10.2527/jas.2011-4237.

López-Gatius, F. (2003). Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. Theriogenology. https://doi.org/10.1016/S0093-691X(02)01359-6.

López-Gatius, F., P. Santolaria, I. Mundet, J. L. Yániz (2005). Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology, 63, 1419-1429

López, H., Z. Wu, L. D. Satter, M. C. Wiltbank (2004). Effect of dietary phosphorus concentration on estrous behaviour of lactating dairy cows. Theriogenology, 61, 437-445.

Lorenzi, T., Meli R, Marzioni D, et al. (2009). Ghrelin: a metabolic signal affecting the reproductive system. Cytokine and Growth Factor Reviews. 20(2):137–152.

Løvendahl, P. and Chagunda, M. G. G. (2010). On the use of physical activity monitoring for estrus detection in dairy cows. Journal of Dairy Science, 93, 249- 259.

Løvendahl, P., Difford, G. F., Li, B., Chagunda, M. G. G., Huhtanen, P., Lidauer, M. H. and Lund, P. (2018). Selecting for improved feed efficiency and reduced methane emissions in dairy cattle. Animal, 12 (s2), s336-s349.

Lucy, M. C. (2008). Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: implications for pp nutrition and reproduction. Reproduction in Domestic Animals 43, Supplement 2, 31–39.

Lucy, M. C. (2012). Growth hormone regulation of follicular growth. Reproduction Fertility and Development. 24 (1) 19–28.

Lucy, M. C. (2019). Symposium review: Selection for fertility in the modern dairy cow—Current status and future direction for genetic selection. Journal of dairy science, 102(4), 3706-3721.

Lucy, M. C. (2001). Reproductive loss in high-producing dairy cattle: where will it end? Journal of Dairy Science, 84(6), 1277–1293.

Lucy, M. C., C. R. Bilby, C. J. Kirby, W. Yuan, and C. K. Boyd (1999). Role of growth hormone in development and maintenance of follicles and corpora lutea. Journal of Reproduction and Fertility. Supplement, vol. 54, pp. 49–59, 1999.

Lucy, M. C., Staples, C. R., Thatcher, W. W., Erickson, P. S., Cleale, R. M., Firkins, J. L., Brodie, B. O. (1992). Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. Animal Science, 54(3), 323–331.

Lyimo, Z.C., M. Nielen, W. Ouweltjes, T.A. Kruip, F.J. van Eerdenburg (2000). Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology, 53, 1783-1795.

McCann, S. M., Kimura, M., Walczewska, A., Karanth, S., Rettori, V., Yu, W. H. (1998). Hypothalamic control of FSH and LH by FSH-RF, LHRH, cytokines, leptin and nitric oxide. NeuroImmunomodulation. 5 (3-4):193–202.

McGee, M., Drennan M. J., Caffrey P. J. (2006). Effect of age and nutrient restriction pre partum on beef suckler cow serum immunoglobulin concentrations, colostrum yield, composition and immunoglobulin concentration and immune status of their progeny. Irish J. Agric. Food Res. 45:157–171.

McGregor, G. P., Desaga, J. F., Ehlenz, K., et al. (1996). Radioimmunological measurement of leptin in plasma of obese and diabetic human subjects. Endocrinology.137 (4):1501–1504.

McMillen, I. C., Adams, M. B., Ross, J. T., Coulter, C. L., Simonetta, G., Owens, J. A., Robinson, J. S., Edwards, L.J. (2001). Fetal growth restriction: adaptations and consequences. Reproduction 122, 195–204.

MacKay, D. and Miller, A. L. (2003). Nutritional support for wound healing. Alternative Medicine Revitas 2003; 8: 359-377.

Madureira, A M L., B F. Silper, T., A. Burnett, L. Polsky, L., H. Cruppe, D., M. Veira, J. L. M., Vasconcelos, R. L. A., Cerr (2015). Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. Journal of Dairy Science, 98 (2015), pp. 7003-7014.

Maillard, V, Froment P, Ramé C, Uzbekova S, Elis S, Dupont J. (2011). Expression and effect of resistin on bovine and rat granulosa cell steroidogenesis and proliferation. Reproduction. 2011;141(4):467–479.

Mann, G. E., Keatinge, R., Hunter, M., Hedley, B. A., and Lamming, G. E. (2005). The use of milk progesterone to monitor reproductive function in beef suckler cows. Animal Reproduction Science, 88(3–4), 169–177.

Mäntysaari, P., J. Juga, M. H. Lidauer, J. Häggman, T. Mehtiö, J. M. Christensen, E. A. Mäntysaari (2022). The relationships between early lactation energy status indicators and endocrine fertility traits in dairy cows. Journal of Dairy Science. 105 (8), 6833-6844.

Mäntysaari, P., A. E. Liinamo, E. A. Mäntysaari (2012) Energy efficiency and its relationship with milk, body, and intake traits and energy status among primiparous Nordic Red dairy cattle. J. Dairy Sci., 95, 3200-3211.

Mäntysaari, P., E. A. Mäntysaari, T. Kokkonen, T. Mehtiö, S. Kajava, C. Grelet, P. Lidauer, M. H. Lidauer (2019). Body and milk traits as indicators of dairy cow energy status in early lactation. J. Dairy Sci., 102, 7904-7916.

Margerison, J. K. (2022). Dairy Cattle. Edition, Eds: Webster, J. and J. Margerison. Management and Welfare of Farm Animals: The UFAW Farm Handbook, 6th Edition. ISBN: 978-1-119-53248-4 June. 59-123.

Margerison, J. K. (2011). Dairy Cattle. United Federation for Animal Welfare (UFAW) Farm Handbook, 5 th Ed. Blackwell Publications. pp 17-47.

Margerison, J. K. and Downey, N. (2005). Guidelines for the nutrition and management of dairy heifers. Edited P. C. Garnsworthy. Nottingham University Press. pp 307-338. ISBN 1-904761-22-4.

Markusfeld, O. (1987). Periparturient traits in seven high dairy herds. Incidence rates, Association with parity, and interrelationships among traits. Journal Dairy Sci.; 70: 158-166.

Mária, M., P. Strapák, I. Szencziová, E. Strapáková and O. Hanušovský (2018). Several methods of estrus detection in cattle dams: A Review," Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 66, (2), 619 – 625.

Marie, M, Findlay, P. A, Thomas, L., Adam, C. L. (2001). Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. Journal of Endocrinology. 170 (1):277–286.

Martin, A. D., N. K. Afseth, A. Kohler, Å. Randby, M. Eknæs, A. Waldmann, G. Dørum, I. Måge, O. Reksen (2015). The relationship between fatty acid profiles in milk identified by Fourier transform infrared spectroscopy and onset of luteal activity in Norwegian dairy cattle. J. Dairy Sci., 98, 5374-5384.

Mantzoros, C. S., D. Qu, R. C. Frederich et al. (1996). Activation of β3 adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. Diabetes, vol. 45, no. 3, pp. 909–914, 1996.

Meikle, A., Kulcsar, M., Chilliard, Y., Febel, H., Delavaud, C., Cavestany, D. and Chilibroste, P. (2004). Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction, 127(6), 727–737.

McClintock, B., K., Gilmour, A. Goddard (2003). Relationships between calving traits in heifers and mature cows in Australia. Interbull Bulletin. 31:102–106.

McDougall, S., Blache, D., and Rhodes, F.M. (2005). Factors affecting conception and expression of oestrus in anoestrous cows treated with progesterone and oestradiol benzoate. Animal Reproduction Science, 88, 203-214.

Micke, G.C., Sullivan, T.M., Magalhaes, R.J.S., Rolls, P.J., Norman, S.T., and Perry, V.E.A. (2010). Heifer nutrition during early- and mid-pregnancy alters fetal growth trajectory and birth weight. Animal Reproduction Science, 117, 1-10.

Miglior, F., A. Fleming, F. Malchiodi, L. F. Brito, P. Martin, C. F. Baes (2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. Journal of Dairy Science, 100 (2017), pp. 10251-10271

Milani, F. X., Nutter, D., and Thoma, G. (2011). Invited review: environmental impacts of dairy processing and products: a review. Journal of Dairy Science, 94(9), 4243–4254.

Miller, G. Y. and C. R. Dorn (1990). Costs of dairy cattle diseases to producers in Ohio. Preventative Veterinary Medicine. 8, 171-182.

Mills, J. A. N.; France, J.; Ellis, J. L.; Crompton, L. A.; Bannink, A.; Hanigan, M. D.; Dijkstra, J. (2017). A mechanistic model of small intestinal starch digestion and glucose uptake in the cow. Journal of Animal Science, 100, 4650–4670.

Minitab 17 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com)

Mohammed, Z. A., Hudson, C. D., Robinson, R. S., and Mann, G. E. (2015). The association between uterine disease and subsequent reproductive performance in commercial UK dairy herds. Society for Reproduction and Fertility Annual Conference 2015, 2. BioScientifica.

Monget, P, Chabrolle C, Dupont J. (2008). Adipose tissue, nutrition and reproduction: what is the link? Bulletin de l'Academie Nationale de Medecine. 192 (4):637–648.

Monget, P. and G. B. Martin (1997). Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals," Human Reproduction, vol. 12, pp. 33–52.

Moore K., Thatcher W. W (2006) Major advances associated with reproduction in dairy cattle. Journal of Dairy Science 89: 1254-1266.

Morrow, D. A., Roberts, S. J., and McEntee, K. (1969). Postpartum ovarian activity and involution of the uterus and cervix in dairy cattle. II. Involution of uterus and cervix. The Cornell Veterinarian, 59 (2), 190–198.

Morton, J. (2001). In-calf project: progress report: a reference for farmers with year-round parturition herds and for their advisers. Dairy Research and Development Corporation, Melbourne, Australia.

Morton, J. (2004). Determinants of reproductive performance of dairy cows in commercial herds in Australia. PhD Thesis. University of Melbourne, Melbourne, Australia. Accessed Oct. 15, 2015.http://hdl.handle.net/11343/38864

Mossa, F., Walsh S.W., Ireland J.J., Evans A.C. (2015). Early nutritional programming and progeny performance: Is reproductive success already set at birth? Animal Frontiers, 5:18–24. doi: 10.2527/af.2015-0004.

Mottet, A., F. Teillard, P. Boettcher, G. De'Besi, B. Besbes (2018). Domestic herbivores and food security: Current contribution, trends and challenges for a sustainable development. Animal, 12, 188-s198.

Mounir, A, Rachid, K, Christian, H, Gary, C. W. (2017). Risk factors of clinical and Subclinical endometritis in cattle: A review. Turkish Journal of Veterinary and Animal Sciences. 2017; 41: 1-11.

Muccioli, G., Lorenzi T., Lorenzi, M., et al. (2011). Beyond the metabolic role of ghrelinn: a new player in the regulation of reproductive function. Peptides. 32(12):2514–2521.

Muñoz-Gutiérrez, M., D. Blache, G. B. Martin, and R. J. Scaramuzzi (2004). Ovarian follicular expression of mRNA encoding the type I IGF receptor and IGF-binding protein-2 in sheep following five days of nutritional supplementation with glucose, glucosamine or lupins. Reproduction, 128 (6) 747–756.

Murney, R. K. Stelwagen, T. T. Wheeler, J. K. Margerison, K. Singh (2015). The effects of milking frequency in early lactation on milk yield, mammary cell turnover, and secretory activity in grazing dairy cows. Journal of Dairy Science. 98 (1), Pages 305-311.

Nadarajah K., Burnside, E. B., Schaeffer, L. R. (1989). Factors affecting gestation length in Ontario Holsteins. Canadian Journal Animal Science. 69:1083–1086.

Nakae, J., Y. Kido, and D. Accili (2001). Distinct and overlapping functions of insulin and IGF-I receptors. Endocrine Reviews, vol. 22, no. 6, pp. 818–835.

Nakazato, M, Murakami N, Date Y, et al. (2001). A role for ghrelin in the central regulation of feeding. Nature. 409(6817):194–198.

Nebel, R. L., Dransfield, M. G., Jobst, S. M., and Bame, J. H. (2000). Automated electronic systems for the detection of oestrus and timing of AI in cattle. Animal Reproduction Science. https://doi.org/10.1016/S0378-4320(00)00090-7.

Negasee, K. A. Clinical Metritis and Endometritis in Diary Cattle: A Review. Veterinary Medicine Open Journal, 5 (2): 51-56.

National Health Service (2022). https://www.nhs.uk/live-well/eat-well/food-types/milk -and-dairy-nutrition/#:~:text=Milk%20and%20dairy%20products% 20are,bones%20and% 20 keep%20teeth%20healthy. Accessed 17/07/2022 11.40.

Nigussie, T. (2018). A review on the role of energy balance on reproduction of dairy cow. Journal of Dairy Research and Technology. DOI:10.24966/DRT-9315/100003

Nilforooshan, M, and Edriss, M. (2004). Effect of age at first calving on some productive and longevity traits in Iranian Holsteins of the Isfahan province. Journal of Dairy Science. 2004;87(7):2130–5.

Nogalski, Z., Piwczyński, D. (2012). Association of length of pregnancy with other reproductive traits in dairy cattle. Asian-Australasian Journal Animal Science 25 (1): 22-7.

Nozière, P.; Ortigues-Marty, I.; Loncke, C.; Sauvant, D. (2010). Carbohydrate quantitative digestion and absorption in ruminants: From feed starch and fibre to nutrients available for tissues. Animal International Journal of Animal Bioscience. 4, 1057–1074.

Nyman, S., Johansson, K., De Koning, D. J., Berry, D. P., Veerkamp, R. F., Wall, E. and Berglund, B. (2014). Genetic analysis of atypical progesterone profiles in Holstein-Friesian cows from experimental research herds. Journal of Dairy Science, 97(11), 7230–7239.

OECD-FAO (2022). Agricultural Outlook 2022-2031. https://doi.org/10.1787/f1b0b29c-en. Accessed 17/07/2-22 11.15.

Ojango, JM, Pollott GE. (2001). Genetics of milk yield and fertility traits in Holstein-Friesian cattle on large-scale Kenyan farms. Journal of Animals, 79: 1742–50.

Omari, M., Lange, A., Plöntzke, J., Röblitz, S. (2020). Model-based exploration of the impact of glucose metabolism on the estrous cycle dynamics in dairy cows. Biology Direct, 15, 2.

Opsomer, G., Coryn, M., Deluyker, H., and de Kruif, A. (1998). An analysis of ovarian dysfunction in high yielding dairy cows after parturition based on progesterone profiles. Reproduction in Domestic Animals, 33 (3-4), 193–204.

Parkinson, T. J. (2019). Infertility in the cow due to functional and management deficiencies, Veterinary Reproduction and Obstetrics, 10, 361-407.

Patton, J., Kenny, D. A., McNamara, S., Mee, J. F., O'mara, F. P., Diskin, M. G., & Murphy, J. J. (2007). Relationships among milk production, energy balance, plasma analytes, and reproduction in Holstein-Friesian cows. Journal of Dairy Science, 90 (2), 649-658.

Pecsok, S. R., McGilliard, M. L., and Nebel, R. L. (2010). Conception Rates. 1. Derivation and Estimates for Effects of Estrus Detection on Cow Profitability. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(94)77242-8.

Pell, J. M., Saunders, J. C., and Gilmour, R. S. (1993). Differential regulation of transcription initiation from insulin-like growth factor-I (IGF-I) leader exons and of tissue IGF-I expression in response to changed growth hormone and nutritional status in sheep. Endocrinology. https://doi.org/10.1210/endo.132.4.8462477.

Pereira, G., P. Simões, R. Bexiga, E. Silva, L. Mateus, T. Fernandes, S. P. Alves Rui, J. B. Bessa, L. Lopes-da-Costa (2022). Effects of feeding rumen-protected linseed fat to postpartum dairy cows on plasma n-3 polyunsaturated fatty acid concentrations and metabolic and reproductive parameters. Journal of Dairy Science**.** 105, (1), 361-374.

Petersson, K. J., B. Berglund, E. Strandberg, H. Gustafsson, A. P. F. Flint, J. A. Woolliams, M. D. Royal (2007). Genetic analysis of postpartum measures of luteal activity in dairy cows. Journal of Dairy Science, 90, 427-434.

Petersson, K. J., H. Gustafsson, E. Strandberg, B. Berglund (2006). Atypical progesterone profiles and fertility in Swedish dairy cows. Journal of Dairy Science. 89 (2006), pp. 2529-2538.

Piccand, V., E. Cutullic, S. Meier, F. Schori, P.L. Kunz, J.R. Roche, P. Thomet (2013). Production and reproduction of Fleckvieh, Brown Swiss, and 2 strains of Holstein-Friesian cows in a pasture-based, seasonal-calving dairy system. Journal of Dairy Science, 96, 5352-5363.

Pirlo, G., Miglior, F., Speroni, M. (2000). Effect of age at first calving on production traits and on difference between milk yield returns and rearing costs in Italian Holsteins. Journal Dairy Science, 83: 603–608.

Plaizier, J. C. B., Lissemore, K. D., Kelton, D., and King, G. J. (2010). Evaluation of Overall Reproductive Performance of Dairy Herds. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(98)75755-8

Pollott, G. E. and Coffey, M. P. (2008). The effect of genetic merit and production system on dairy cow fertility, measured using progesterone profiles and on-farm recording. Journal of Dairy Science, 91, 3649-3660.

Pond, W.G., Maurer, R.R., Mersmann, H., Cummins, S. (1992). Response of fetal and newborn piglets to maternal protein restriction during early or late pregnancy. Growth Development and Aging, 56 (3), 115–127.

Poppitt, S. D. (2020). Cow's Milk and Dairy Consumption: Is There Now Consensus for Cardiometabolic Health? Front. Nutr., Sec. Nutrition and Metabolism. 7, 1-22. https://doi.org/10.3389/fnut.2020.574725. Accessed 17/07/2022. 11.55

Poretsky, L, Cataldo NA, Rosenwaks Z, Giudice LC. (1999). The insulin-related ovarian regulatory system in health and disease. Endocrine Reviews, 20 (4):535–582.

Potts, S.B., M. Shaughness, R.A. Erdman (2017). The decline in digestive efficiency of US dairy cows from 1970 to 2014. Journal of Dairy Science, 100, pp. 1-11.

Prunier, A. and H. Quesnel (2000). Nutritional influences on the hormonal control of reproduction in female pigs. Livestock Production Science, 63 (1), 1–16.

Pryce, J. E., Coffey, M. P., Brotherstone, S. H. and Woolliams, J. A. (2002). Genetic relationships between parturition interval and body condition score conditional on milk yield. Journal of Dairy Science, 85, 1590–1595.

Pryce, J. E., Nielsen, B. L., Veerkamp, R. F. and Simm, G. (1999). Genotype and feeding system effects and interactions for health and fertility traits in dairy cattle. Livestock Production Science, 57 (3), 193–201.

Pryce, J. E., Veerkamp, R. F., Thompson, R., Hill, W. G. and Simm, G. (1997). Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. Animal Science, 65 (3), 353-360.

Pryce, J. E., & Veerkamp, R. F. (2001). The incorporation of fertility indices in genetic improvement programmes. BSAP Occasional Publication, 26 (1), 237-249.

Purcell, S. H. and K. H. Moley (2009). Glucose transporters in gametes and preimplantation embryos. Trends in Endocrinology and Metabolism, vol. 20, no. 10, pp. 483–489, 2009.

Purcell, S. H. and K. H. Moley (2011). The impact of obesity on egg quality. Journal of Assisted Reproduction and Genetics. 28, 517–524.

Pushpakumara, P. G. A., Robinson, R. S., Demmers, K. J., Mann, G. E., Sinclair, K. D., Webb, R., and Wathes, D. C. (2002). Expression of the insulin-like growth factor (IGF) system in the bovine oviduct at oestrus and during early pregnancy. Reproduction-Cambridge-, 123(6), 859–868.

Pyman, M. F. (2006). Dairy crossbreeding to improve health and reproductive performance. Animal Production and Animal Science Worldwide, 263.

Quennell, JH, Howell CS, Roa J, Augustine RA, Grattan DR, Anderson GM. (2011). Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinology. 2011;152(4):1541–1550.

Quesnel, H., A. Pasquier, A. M. Mounier, and A. Prunier. (2002). Feed restriction in cyclic gilts: gonadotrophin-independent effects on follicular growth, Reproduction Nutrition Development, vol. 40, no. 4, pp. 405–414, 2000.

Rae, M.T., Kyle, C.E., Miller, D.W., Hammond, A.J., Brooks, A.N., and Rhind, S.M. (2002). The effects of undernutrition, in utero, on reproductive function in adult male and female sheep. Animal Reproduction Science, 72, 63-71.

Rae, M. T., S. Palassio, C. E. Kyle et al (2001). Effect of maternal undernutrition during pregnancy on early ovarian development and subsequent follicular development in sheep fetuses," Reproduction, 122 (6) 915–922.

Rae, M. T., S. M. Rhind, P. A. Fowler, D. W. Miller, C. E. Kyle, and A. N. Brooks (2002). Effect of maternal undernutrition on fetal testicular steroidogenesis during the CNS androgen-responsive period in male sheep fetuses. Reproduction, 124 (1) 33–39.

Ramaley, J. A. and C. K. Phares (1980). Delay of puberty onset in females due to suppression of growth hormone. Endocrinology, vol. 106, no. 6, pp. 1989–1993.

Redmer, D. A., Wallace, J. M., Reynolds, L. P. (2004). Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domesticated Animal Endocrinology, 27, 199–217.

Reist, R., A. Koller, A. Busato, U. Küpfer, J. W. Blum (2000). First ovulation and ketone body status in the early postpartum period of dairy cows. Theriogenology, 54, 685-701.

Reith, S., H. Brandt, S. Hoy (2014). Simultaneous analysis of activity and rumination time, based on collar-mounted sensor technology, of dairy cows over the peri-estrus period. Livestock Science, 170 (2014), pp. 219-227.

Reynolds, C. K., Crompton, L. A. and Mills, J. A. N. (2011). Improving the efficiency of energy utilisation in cattle, Animal Production Science. 51 (1), 6–12.

Reynolds, C. K.; Humphries, D. J.; van Vuuren, A. M.; Dijkstra, J.; Bannink, A. (2014). Considerations for feeding starch to high-yielding dairy cows. In Proceedings of the 46th University of Nottingham Feed Conference, Nottingham, UK, 24–26; pp. 9–11. 41.

Rhind, S. M. (2004). Effects of maternal nutrition on fetal and neonatal reproductive development and function. Animal Reproduction Science, 82-3, 169-181.

Rhind, S. M., D. A. Elston, J. R. Jones, M. E. Rees, S. R. McMillen, and R. G. Gunn (1998). Effects of restriction of growth and development of Brecon Cheviot ewe lambs on subsequent lifetime reproductive performance. Small Ruminant Research, 30 (2) 121–126.

Rhind, S. M., S. R. Mcmillen, E. Duff, D. Hirst, and S. Wright (1998), Seasonality of meal patterns and hormonal correlates in red deer Physiology and Behavior, 65 (2), 295–302.

Rhind, S. M., S. McMillen, W. A. C. McKelvey, F. F. Rodriguez-Herrejon, and A. S. McNeilly (1989). Effect of the body condition of ewes on the secretion of LH and FSH and the pituitary response to gonadotrophin-releasing hormone. Journal of Endocrinology, 120 (3) 497–502.

Roberts, S. J. (1971). Veterinary obstetrics and genital diseases (Theriogenology).

Robinson, J. J., Ashworth, C. J., Rooke, J. A., Mitchell, L. M., and McEvoy, T. G. (2006). Nutrition and fertility in ruminant livestock. Animal Feed Science and Technology, 126, 259-276.

Roche, J. F., Bolandl, M. P., and McGeady, T. A. (1981). Reproductive wastage following artificial insemination of heifers. The Veterinary Record, 109(18), 401–404.

Roche, J. R., Friggens, N. C., Kay, J. K., Fisher, M. W., Stafford, K. J., and Berry, D. P. (2009). Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. Journal Dairy Science, 92 (12), 5769–5801. https://doi.org/10.3168/jds.2009-2431

Rodriguez-Pacheco, F., Martinez-Fuentes, A.J., Tovar, S., et al. (2007). Regulation of pituitary cell function by adiponectin. Endocrinology. 148 (1):401–410.

Roelofs, J., López-Gatius, F., Hunter, R. H. F., van Eerdenburg, F. J. C. M., and Hanzen, C. (2010). When is a cow in estrus? Clinical and practical aspects. Theriogenology, 74(3), 327–344. https://doi.org/10.1016/J.THERIOGENOLOGY.2010.02.016.

Rodney, R. M., Celi P., Scott W., Breinhild K., Lean I. (2015). Effects of dietary fat on fertility of dairy cattle: A meta-analysis and meta-regression. Journal of Dairy Science. 98 (26094218): 5601-5620.

Roland, A. V. and S. M. Moenter, (2011). Regulation of gonadotropin-releasing hormone neurons by glucose, Trends in Endocrinology and Metabolism, 22 (11), 443–449.

Roth, Z., Arav, A., Bor, A., Zeron, Y., Braw-Tal, R., and Wolfenson, D. (2001). Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction-Cambridge, 122 (5), 737–744.

Royal, M. D., Darwash, A. O., Flint, A. P. F., Webb, R., Woolliams, J. A., and Lamming, G. E. (2000). Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Animal Science, 70(3), 487–501.

Royal, M.D. J. Pryce, J. Woolliams, A. Flint (2002). The genetic relationship between commencement of luteal activity and calving interval, body condition score, production, and linear type traits in Holstein-Friesian. Journal of Dairy Science. 85 3071-3080.

Royal, M. D., A.P.F. Flint, J.A. Woolliams (2002). Genetic and phenotypic relationships among endocrine and traditional fertility traits and production traits in Holstein-Friesian dairy cows. Journal of Dairy Science. 85, 958-967.

Royal, M.D., Smith, R.F., and Friggens, N.C. (2008). Fertility in dairy cows: bridging the gaps - Foreword. Animal, 2, 1101-1103.

Ruiz-Cortés, Z. T, Martel-Kennes Y, Gévry, N.Y, Downey B.R., Palin MF, Murphy B.D. (2003). Biphasic effects of leptin in porcine granulosa cells. Biology of Reproduction. 68 (3):789–796.

Rumphorst, T., Scheu, T., Koch, C., & Sundrum, A. (2022). Inter-and Intra-Individual Variation in the Behavior of Feed Intake on Nutrient Availability in Early Lactating Dairy Cows. Animals, 12 (1), 37.

Rutledge, J. J., Monson, R. L., Northey, D. L., and Leibfried-Rutledge, M. L. (1999). Seasonality of cattle embryo production in a temperate region. Theriogenology, 51(1), 330.

Sagae, S. C., E. F. Menezes, M. L. Bonfleur et al. (2012). Early onset of obesity induces reproductive deficits in female rats. Physiology and Behavior, 105 (5) 1104–1111.

Sherwin, V. E., Hudson, C. D., Henderson, A., Green, M. J. (2016). The association between age at first calving and survival of first lactation heifers within dairy herds. Animal. 10: 1877–1882.

Sundrum, A. Metabolic Disorders in the Transition Period Indicate that the Dairy Cows' Ability to Adapt is Overstressed. Animals, 5, 978–1020.

Sanchez, J. M, Mathew, D. J, Passaro, C, Fair T, Lonergan, P. (2018) Embryonic maternal interaction in cattle and its relationship with fertility. Reproduction of Domestic Animals. 53:20–27.

Sartori, R, Sartor-Bergfelt, R., Mertens, S. A., Guenther, J. N., Parrish, J. J., and Wiltbank, M. C. (2002). Fertilization and early embryonic development in heifers and lactating cows

in summer and lactating and dry cows in winter. Journal of Dairy Science, 85(11), 2803–2812.

Sartori, Roberto, Bastos, M. R., and Wiltbank, M. C. (2009). Factors affecting fertilisation and early embryo quality in single-and superovulated dairy cattle. Reproduction, Fertility and Development, 22(1), 151–158.

Sauer, M. J., Foulkes, J. A., Worsfold, A., and Morris, B. A. (2004). Use of progesterone 11-glucuronide--alkaline phosphatase conjugate in a sensitive microtitre-plate enzymeimmunoassay of progesterone in milk and its application to pregnancy testing in dairy cattle. Reproduction. https://doi.org/10.1530/jrf.0.0760375.

Scaramuzzi, R. J., H. M. Brown, and J. Dupont (2010). Nutritional and metabolic mechanisms in the ovary and their role in mediating the effects of diet on folliculogenesis: a perspective. Reproduction in Domestic Animals, 45 (3) 32–41.

Scaramuzzi, R. J., B. K. Campbell, J. A. Downing et al. (2006). A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reproduction Nutrition Development, vol. 46, no. 4, pp. 339–354.

Scaramuzzi, R. J., J. F. Murray, J. A. Downing, and B. K. Campbell (1999). The effects of exogenous growth hormone on follicular steroid secretion and ovulation rate in sheep. Domestic Animal Endocrinology, vol. 17, no. 2-3, pp. 269–277.

Schneider, J. E. (2004). Energy balance and reproduction, Physiology and Behavior, 81, (2), 289–317.

Short, R. E. and R. A. Bellows (1971). Relationships among weight gains, age at puberty and reproductive performance in heifers," Journal of Animal Science, 32, 127–131.

Shrestha, H. K., Nakao, T., Suzuki, T., Akita, M. and Higaki, T. (2005). Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. Theriogenology, 64 (4), 855–866.

Silper, B. F., A. M. L., Madureira, M. Kaur, T. A. Burnett, R L A Cerri (2015). Short communication: Comparison of estrus characteristics in Holstein heifers by 2 activity monitoring systems. Journal of Dairy Science, 98 3158-3165.

Silva, H. M., Wilcox C. J., Thatcher, W. W., Becker, R. B., Morse, D. (1992). Factors affecting days open, gestation length, and calving interval in Florida dairy cattle. Journal Dairy Science, 75:288–293.

Simmons, R.A., Flozak, A.S., Ogata, E.S. (1993). The effect of insulin and insulin-like growth factor-I on glucose transport in normal and small for gestational age foetal rats. Endocrinology, 133, 1361–1368.

Sjaunja, L. O., Baevre, L., Junkkarinen, L., Pedersen, J., and Setälä, J. (1990). A Nordic proposal for an energy corrected milk formula. Proc. 2nd Session of Committee for Recording and Productivity of Milk Animal Paris, 156.

Smith, B. I, Risco C. A. (2002). Therapeutic and management options for postpartum metritis in dairy cattle. Composition of Continuing Education Practice Veterinary .; 24: 92-100.

Spalding, R., Everett R., Foote R. (1975). Fertility in New York artificially inseminated Holstein herds in dairy herd improvement. Journal of Dairy Science. 58:718-723.

Spencer, T. E., Forde, N., Dorniak, P., Hansen, T. R., Romero, J. J., Lonergan, P. (2013) Conceptus-derived prostaglandins regulate gene expression in the endometrium prior to pregnancy recognition in ruminants. Reproduction 146:377–387.

Spicer, L. J. (2001). Leptin: a possible metabolic signal affecting reproduction. Domestic Animal Endocrinology. 2001;21(4):251–270.

Spicer, L. J., Francisco, C. C. (1997). The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. Endocrinology. 138 (8):3374–3379.

Spicer, LJ, Schreiber NB, Lagaly DV, Aad PY, Douthit LB, Grado-Ahuir JA. (2011). Effect of resistin on granulosa and theca cell function in cattle. Animal Reproduction Science. 124 (1-2):19–27.

Taylor, V. J., Beever, D. E., Bryant, M. J. and Wathes, D. C. (2003). Metabolic profiles and progesterone cycles in first lactation dairy cows. Theriogenology, 59 (7), 1661–1677.

Taylor, V. J., Cheng, Z., Pushpakumara, P. G. A., Beever, D. E., and Wathes, D. C. (2004). Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. Veterinary Record. https://doi.org/10.1136/vr.155.19.583.

Taylor, R. K., LeMaster, C. T., Mangrum, K. S., Ricks, R. E., Long, N. M. (2018). Effects of maternal nutrient restriction during early or mid-gestation without realimentation on maternal physiology and foetal growth and development in beef cattle. Animal. 12:312–321.

Taylor, A. R., Mohrhauser, D. A., Pritchard, R. H., Underwood, K. R., Wertz-Lutz, A. E., Blair, A.D. (2016). The influence of maternal energy status during mid-gestation on growth, cattle performance, and the immune response in the resultant beef progeny. Professional Animal Science, 32:389–399.

Tena-Sempere, M. (2006). GPR54 and kisspeptin in reproduction. Human Reproduction Update. 12 (5):631–639.

Tena-Sempere, M. (2007). Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology. 86 (3):229–241.

Tersigni, C, Di Nicuolo F, D'Ippolito S, Veglia M, Castellucci M, Di Simone N. (2011). Adipokines: new emerging roles in fertility and reproduction. Obstetrical and Gynecological Survey. 2011;66(1):47–63.

Thatcher, W. W., Bartol, F. F., Knickerbocker, J. J., Curl, J. S., Wolfenson, D., Bazer, F. W., and Roberts, R. M. (1984). Maternal recognition of pregnancy in cattle. Journal of Dairy Science, 67(11), 2797–2811.

Thatcher, W., Bilby T., Bartolome J., Silvestre F., Staples C., Santos J. (2006). Strategies for improving fertility in the modern dairy cow. Theriogenology., 65: 30-44.

The Feeding Stuffs (England) Regulations 2005, Statutory Instrument No. 3281, Her Majesty's Stationery Office, London (2005).

Thomassen, M. A., van Calker, K. J., Smits, M. C. J., Iepema, G. L., and de Boer, I. J. M. (2008). Life cycle assessment of conventional and organic milk production in The Netherlands. Agricultural Systems, 96, 95–107.

Toledo-Alvarado, H., A. I. Vazquez, G. de los Campos, R. J. Tempelman, G. Gabai, A. Cecchinato, G. Bittante (2018). Changes in milk characteristics and fatty acid profile during the estrous cycle in dairy cows. Journal of Dairy Science, 101 (10) 9135-9153.

Toledo, I.M, X. Zhao and P. Lacasse (2020). Effects of milking frequency and domperidone injections on milk production and prolactin signaling in the mammary gland of dairy cows. Journal of Dairy Science. 103 (2), Pages 1969-198.

Turk R, Samardzija M, Bacic G. (2011). Oxidative stress and reproductive disorders in dairy cows. In: Marek ER, ed. Dairy Cows: Nutrition, Fertility, and Milk Production. New York, USA: Nova Science Publishers: 57-98.

United Nations (2022) Climate action. https://www.un.org/en/climatechange/science/key-findings#:~:text=The%20emissions%20gap%20in%202030,to%20below%202%C2%B0C. Accessed 17/07/2022. 12.25.

Valenza, A., J. O. Giordano, G. Lopes, Jr, L. Vincenti, M. C. Amundson, P. M. Fricke (2012). Assessment of an acceleration system for detection of estrus and treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows. Journal of Dairy Science, 95, pp. 7115-7127.

Van Binsbergen, R., Bouwman, A. C., and Veerkamp, R. F. (2019). Genetic parameters for atypical reproductive patterns in dairy cows estimated from in-line milk progesterone profiles. Journal of Dairy Science, 102(12), 11104–11115.

Vandehaar, M.J., L.E. Armentano, K. Weigel, D.M. Spurlock, R.J. Tempelman, R. Veerkamp (2016). Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. Journal of Dairy Science, 99, 4941-4954.

VandeHaar, M. J. and St-Pierre, N. (2006). Major advances in nutrition: relevance to the sustainability of the dairy industry, Journal of Dairy Science. 89(4), 1280–1291.

Vanroose, G., de Kruif, A., and Van Soom, A. (2000). Embryonic mortality and embryo-pathogen interactions. Animal Reproduction Science. 60, 131-143.

Veerkamp, R.F., J.K. Oldenbroek, H.J. van der Gaast, J.H.J. van der Werf (2000). Genetic correlation between days until start of luteal activity and milk yield, energy balance, and live weights. Journal of Dairy Science. 83, 577-583.

Vellinga, T. V., Blonk, H., Marinussen, M., Van Zeist, W. J. and Starmans, D. A. J. (2013). Methodology used in feedprint: a tool quantifying greenhouse gas emissions of feed production and utilization (No. 674). Wageningen UR Livestock Research.

Verkerk, G. a, Morgan, S., and Kolver, E. S. (2000). Comparison of selected reproductive characteristics in Overseas and New Zealand Holstein-Friesian cows grazing pasture or fed a total mixed ration. New Zealand Society of Animal Production.

Vicini, J. L., Buonomo, F. C., Veenhuizen, J. J., Miller, M. A., Clemmons, D. R., and Collier, R. J. (1991). Nutrient balance and stage of lactation affect responses of insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatotropin administration in dairy cows. The Journal of Nutrition, 121 (10), 1656–1664.

Vizcarra, J. A., R. P. Wettemann, J. C. Spitzer, and D. G. Morrison (1998). Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin, and nonesterified fatty acids in plasma of primiparous beef cows. Journal of Animal Science, vol. 76, no. 4, pp. 927–936.

von Soosten, D., Meyer U., Piechotta M., Flachowsky G., Dänicke S. (2012). Effect of conjugated linoleic acid supplementation on body composition, body fat mobilization, protein accretion, and energy utilization in early lactation dairy cows. Journal of Dairy Science. 95: 1222-1239.

Wallace, J. M., R. P. Aitken, and M. A. Cheyne (1996). Nutrient partitioning and fetal growth in rapidly growing adolescent ewes, Journal of Reproduction and Fertility, vol. 107, no. 2, pp. 183–190, 1996.

Walsh, S. W., Williams, E. J., and Evans, A. C. O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science, 123(3–4), 127–138.

Walsh, S.W., Williams, E.J. and Evans, A.C.O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science123, 127–138.

Warrington, B. G., Byers, F. M., Schelling, G. T., Forrest, D. W., Baker, J. F., Greene, L. W. (1988). Gestation nutrition, tissue exchange and maintenance requirements of heifers. Journal of Animal Science. Sci. 66, 774–782.

Wathes, D. C. (2012). Mechanisms linking metabolic status and disease with reproductive outcome in the dairy cow. Reproduction in Domestic Animals, 47 Supplement 4, 304-312.

Wathes, D. C., Brickell, J. S., Bourne, N. E., Swali, A., Cheng, Z. (2008). Factors influencing heifer survival and fertility on commercial dairy farms. Animal. 2, 1135–43.

Wathes, D., Pollott, G., Johnson, K., Richardson, H. and Cooke, J. (2014). Heifer fertility and carry over consequences for lifetime production in dairy and beef cattle. Animal. 8 (s1):91–104.

Wang, Q., M. M. Chi, T. Schedl et al. (2012). An intercellular pathway for glucose transport into mouse oocytes," American Journal of Physiology-Endocrinology and Metabolism, 302 (12), E1511–E1518.

Webb, R., Garnsworthy, P. C., Gong, J. G. and Armstrong, D. G. (2004). Control of follicular growth: Local interactions and nutritional influences. Journal of Animal Science, 82, 63-74.

Webb, R., R. G. Gosden, E. E. Telfer, and R. M. Moor (1999). Factors affecting folliculogenesis in ruminants. Animal Science, 68 (2) 257–284.

Webb, R., B. Nicholas, J. G. Gong et al. (2003). Mechanisms regulating follicular development and selection of the dominant follicle. Reproduction Supplement, 61, 71–90.

Webster, J. and J. Margerison (2022). Management and Welfare of Farm Animals: The UFAW Farm Handbook, 6th Edition. ISBN: 978-1-119-53248-4 June 2022 Wiley-Blackwell

Westwood, C. T., Lean, I. J., and Garvin, J. K. (2010). Factors Influencing Fertility of Holstein Dairy Cows: A Multivariate Description. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(02)74411-1.

Wientjes, J. G. M., N. M. Soede, H. Van den Brand, and B. Kemp (2012). Nutritionally induced relationships between insulin levels during the weaning-to-ovulation interval and reproductive characteristics in multiparous sows: I. luteinizing hormone, follicle development, oestrus and ovulation. Reproduction in Domestic Animals, vol. 47, no. 1, pp. 53–61.

Wientjes, J. G. M., N. M. Soede, F. Aarsse et al. (2012). Effects of dietary carbohydrate sources on plasma glucose, insulin and IGF-I levels in multiparous sows. Journal of Animal Physiology and Animal Nutrition, 96 (3) 494–505.

Williams, S. A., D. Blache, G. B. Martin, R. Foot, M. A. Blackberry, and R. J. Scaramuzzi (2001). Effect of nutritional supplementation on quantities of glucose transporters 1 and 4 in sheep granulosa and theca cells. Reproduction, vol. 122, no. 6, pp. 947–956.

Windig, J.J., B. Beerda, R. Veerkamp (2008). Relationship between milk progesterone profiles and genetic merit for milk production, milking frequency, and feeding regimen in dairy cattle. Journal of Dairy Science. 91, 2874-2884.

Wolf, P., Groen, E. A., Berg, W., Prochnow, A., Bokkers, E. A., Heijungs, R., and de Boer, I. J. (2017). Assessing greenhouse gas emissions of milk production: which parameters are essential? The International Journal of Life Cycle Assessment, 22 (3), 441-455.

Yan, T., R.E. Agnew, F.J. Gordon, M.G. Porter (2000). Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. Livestock Production Science, 64, 253-263.

Yates, C.M., Cammell, S.B., France, J., Beever, D.E. (2000). Prediction of methane emissions from dairy cows using multiple regression analysis. Proceeding of the British Society of Animal Science. (Vol. 2000, pp. 94-94). Cambridge University Press.

Yelich, J. V., R. P. Wettemann, T. T. Marston, and L. J. Spicer, (1996) Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates, Domestic Animal Endocrinology, 13 (4) 325–338.

Yan, J., B. Zhou, J. Yang et al. (2008). Glucose can reverse the effects of acute fasting on mouse ovulation and oocyte maturation. Reproduction, Fertility and Development, 20 (6) 703–712.

Yang, X., L. L. Wu, L. R. Chura et al. (2012), Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. Fertility and Sterility, 97 (6) 1438–1443, 2012.

Yi, D., S. Zeng, and Y. Guo (2012). A diet rich in n-3 polyunsaturated fatty acids reduced prostaglandin biosynthesis, ovulation rate, and litter size in mice. Theriogenology, 78 (1), 28–38.

Youngquist, R. S and Shore, O. (1997). Postpartum uterine infections. In: Youngquist, R. S, ed. Current Therapy in Large Animal Theriogenology. Toronto, Canada: WB Saunders Company. 456.

Yu, W. H., Kimura, M., Walczewska, A., Karanth, S., McCann, S. M. (1997). Role of leptin in hypothalamic-pituitary function. Proceedings of the National Academy of Sciences of the United States of America. 94:1023–1028.

Xu, Z. Z., McKnight, D. J., Vishwanath, R., Pitt, C. J., and Burton, L. J. (2010). Estrus Detection Using Radiotelemetry or Visual Observation and Tail Painting for Dairy Cows on Pasture. Journal of Dairy Science. https://doi.org/10.3168/jds.s0022-0302(98)75849-7.

Zago, D., Canozzi MEA, Barcellos JOJ. (2020). Pregnant beef cow's nutrition and its effects on postnatal weight and carcass quality of their progeny. PLoS One. 2020 Aug 27;15(8):e0237941. doi:

Zambrano, E., Rodriguez-Gonzalez, G.L., Guzman, C., Garcia-Becerra, R., Boeck, L., Diaz, L., Menjivar, M., Larrea, F., and Nathanielsz, P.W. (2005). A maternal low protein diet

during pregnancy and lactation in the rat impairs male reproductive development. Journal of Physiology-London, 563, 275-284.

Zhang, C., Juniper, D. T. and Meagher, R. K. (2022). Effects of physical enrichment and pair housing before weaning on growth, behaviour and cognitive ability of calves after weaning and regrouping. Applied Animal Behaviour Science, 249, 105606.

Zavadilová L and Štípková M. (2013). Effect of age at first calving on longevity and fertility traits for Holstein cattle. Czech Journal Animal Science. 2013;58(2):47–57.

Zehetmeier, M., Hoffmann, H., Sauer, J., Hofmann, G., Dorfner, G., and O'Brien, D. (2014). A dominance analysis of greenhouse gas emissions, beef output and land use of German dairy farms. Agricultural Systems, 129, 55-67.

Zulu, V. C., Sawamukai, Y., Nakada, K., Kida, K., and Moriyoshi, M. (2002). Relationship among Insulin-Like Growth Factor-I, Blood Metabolites and Pp Ovarian Function in Dairy Cows. Journal of Veterinary Medical Science. https://doi.org/10.1292/jvms.64.879.